

IN-52-CR

72173

P-90

Final Report

"PATHOPHYSIOLOGY OF SPONTANEOUS VENOUS GAS EMBOLISM"

Project NAG9-154 S5

To

Life Sciences Division
and
Johnson Space Center

National Aeronautics and Space Administration

C. J. Lambertsen, M.D.
Institute For Environmental Medicine
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

10 November 1991

(NASA-CR-189915) PATHOPHYSIOLOGY OF
SPONTANEOUS VENOUS GAS EMBOLISM Final Report
(Pennsylvania Univ.) 90 p CSCL 06P

N92-19761

Unclassified
G3/52 0072173

"Pathophysiology of Spontaneous Venous Gas Embolism"

Contents

1.0 ABSTRACT

2.0 INTRODUCTION AND BACKGROUND

 2.1 Circumstances of Venous Gas Embolism, Hyperbaric Decompression,
 Hypobaric Decompression, Isobaric Counterdiffusion, Air Infusion

 2.2. Pathologic Consequence of Decompression

 2.3. Isobaric Counterdiffusion
 Production of Gas Lesion Disease
 Use as Investigative Model for Venous Gas Embolism

 2.4 Contrast With Venous Air Infusion Embolism

3.0 GOALS AND SCOPE OF PROJECT

 3.1 Initial Objectives

 3.2 Modification of Scope

4.0 METHODS OF VENOUS GAS EMBOLISM EXPOSURE AND MEASUREMENTS

 4.1 Counterdiffusion Generation in Pigs

 4.2 Monitoring and Measurements For Cardiovascular/Respiratory Effects

 4.3 Sampling and Measurements of Blood

 4.4 Pulmonary Microcirculation Histologic Examination

5.0 PATHOPHYSIOLOGIC EFFECTS OF VENOUS GAS EMBOLISM

 5.1 Exposure, Whole Body, Lethal Counterdiffusion

 5.2 Effects on Blood Systems

 5.3 Effects on Lung Gross and Pulmonary Microstructure

 5.4. Contrast of Air Infusion With Counterdiffusion Venous Gas Embolism

6.0 SUMMARY INTERPRETATIONS

7.0 REFERENCES

8.0 FIGURES

9.0 APPENDICES

- A. Document. "Relation of Isobaric Inert Gas Counterdiffusion and Decompression Gas Lesion Diseases."
- B. Draft: "Cardiovascular and Respiratory Effects of Isobaric Counterdiffusion Venous Gas Embolism."
- C. Draft: "Effects of Isobaric Counterdiffusion Venous Gas Embolism on Blood Contact System."
- D. Document. Summary of Prior Study: "Infusion of Air Emboli in Sheep. Effects on Pulmonary Microvascular Ultrastructure. Relation to Pulmonary Lymph Flow."

Participants

C.J. Lambertsen, M.D.
Principal Investigator

K.H. Albertine, Ph.D.
Co-principal Investigator

J. B. Pisarello, M.D.

N.D. Flores, M.D.

1.0 ABSTRACT SUMMARY "PATHOPHYSIOLOGY OF SPONTANEOUS VENOUS GAS EMBOLISM"

The discovery of the phenomenon of "Superficial Isobaric Inert Gas Counterdiffusion Supersaturation" interposed a new and lethal gas lesion disease between the decompression sicknesses of underwater and aerospace activity. All three include venous gas embolization as well as generation of gas lesions in peripheral sites of origin of gas phase, and damage to tissues in which gas lesions form. Whereas the sites, timing and duration of gas lesions in the two decompression-related conditions are unpredictable, gas lesion location and degree in isobaric counterdiffusion are controllable, as is the degree and duration of its stable rate of continuous venous gas embolism.

This program concerned the use of controllable degrees and durations of continuous isobaric counterdiffusion venous gas embolism to investigate effects of venous gas embolism upon blood, cardiovascular, and respiratory gas exchange function, as well as pathological effects upon the lung and its microcirculation.

Use of N₂O/He counterdiffusion permitted performance of the pathophysiologic and pulmonary microstructural effects at one ATA without hyperbaric or hypobaric exposures.

One component of the program entailed tracking of pathophysiologic effects of venous gas embolism leading to death. In a shorter duration of the same degree of continuous venous gas embolism examination was made for effects of blood/gas phase interactions on blood contact system. A lesser degree of multi-hour pulmonary venous gas embolism was used to investigate pathologic effects of isobaric venous gas embolism upon lung microstructure.

An initial intent of the program included determination of interaction between pulmonary damage by venous gas embolism and pulmonary effects of oxygen poisoning, as this might relate to hyperbaric therapy of severe decompression sickness.

Continuous venous gas embolism associated with whole body (head and neck excluded) counterdiffusion of artificially respiration animals was tolerated for 1 to 4 hours without major cardiovascular or hematologic effects, except for early death in two animals which had patent interatrial septal defects. Death of other animals after prolonged counterdiffusion and venous gas embolization was associated with hemoconcentration, plasma loss, hypotension, metabolic and respiratory acidosis, and arterial hypoxia and hypercapnia in the face of constant artificial ventilation.

Absence of detectable pulmonary damage by the venous gas emboli of isobaric counterdiffusion made study of interaction of interaction with therapeutic degrees of oxygen poisoning not practical. An alternative investigation established that intra-atrial continuous infusion of air bubbles did produce pulmonary microvascular injury in pigs whereas the isobaric counterdiffusion venous gas embolization did not.

2.0 INTRODUCTION AND BACKGROUND

Venous Gas Embolism (VGE) directly relevant to NASA missions occurs in human subjects exposed to ambient pressure reduction in hypobaric chambers, in simulation of operational hypoobaric profiles associated with extra-vehicular activity (EVA) in the space environment, in high altitude aviation, and in decompression from prolonged exposures to compressed air breathing in underwater neutral buoyancy simulation. The classical procedure of oxygen breathing has been extensively investigated to accelerate nitrogen elimination and prevent venous gas embolism and symptoms of hypobaric decompression sickness (11)(22).

The long (multi-hour) periods of oxygen breathing required to prevent decompression sickness and/or venous gas embolism impose considerable operational restriction, as well as potential cumulative effects of oxygen, especially in relation to daily repetitive EVA from a one atmosphere space compartment (11)(22).

Since venous gas embolism can occur without symptomatic expression of decompression sickness, it has been necessary to determine the characteristics and significance of any physiologic or pathophysiologic effects produced by the gas embolic process. In such investigation it is also necessary to distinguish among several different forms of venous gas embolism used in experimental procedures (14).

2.1 Forms of Venous Gas Embolization (VGE)

Common features of operational or experimental venous gas embolization include any effects upon blood itself, physiologic changes effects on lung tissue or pulmonary vasculature, and potential for transpulmonary embolism of the arterial system and the entire body system it supplies.

Important differences among the several recognized forms of venous gas embolism, each encountered operationally and used experimentally, include:

(a) Decompression from transient (non-saturating) hyperbaric exposures (e.g. Diving). All body tissues are exposed to increased inert gas pressures during hyperbaria, to different degrees in myriad sites. Improper decompression procedures can presumably produce VGE from any or all undefinable foci of generation (14). VGE formation is transient and unpredictable in onset, degree or duration. Effects of such gas emboli are therefore unpredictable pulmonary arterial blood, derived from the venous drainage of all tissue sites, is supersaturated unless specific measures (e.g. oxygen breathing) are taken to prevent this.

(b) Intentional or accidental infusion of air into venous blood stream (e.g. experiment or clinical procedure). Body tissues and venous blood normally have sub-atmospheric total gas pressure. Bubbles of infused venous gas emboli, not having generated in peripheral tissues, do not produce products of peripheral tissue damage.

(c) Decompression from normal air breathing at one atmosphere to hypobaric states (e.g. altitude exposure, chamber experiment, EVA) (11). Decompression is from a state of nitrogen saturation of all tissues, including tissues with slowest rates of gas elimination. This circumstance is dynamically equivalent to a decompression from hyperbaric nitrogen saturation (as in ascent from multi-day shallow air saturation diving). VGE is prolonged, but unpredictable in onset, degree or duration.

(d) Deep Tissue Isobaric Inert Gas Counterdiffusion (e.g. breathing different inert gases in sequence) (14)(8). VGE are generated when helium is breathed at high ambient pressures following prolonged exposure to nitrogen-oxygen atmospheres. Gas supersaturation is transient, in all tissues. VGE onset, degree, duration and sites of origin are unpredictable.

(e) Superficial Isobaric Inert Gas Counterdiffusion (e.g. Breathing air by mask in a helium-filled diving bell). Supersaturation of superficial skin tissues without compression or decompression (isobaric state) leads to dermal and subcutaneous gas lesions, and venous gas emboli (7)(12)(14)(15)(19). In the stable counterdiffusion environmental situation the volume rate of venous gas embolism begins early, continues at an essentially uniform stable rate throughout the counterdiffusion exposures, with its degree proportional to the exposed surface area. Specific microsites of gas emboli are not known. Venous blood delivered to pulmonary arterial circulation comprises venous blood of unsaturated inert gas composition from all deep tissues, mixed with supersaturated blood from the counterdiffused superficial tissue. The inert gas composition of the mixed venous blood is therefore not precisely predictable, any more than it is in decompression (14).

2.2 Isobaric Counterdiffusion As An Experimental Model for VGE Pathophysiology

Superficial Isobaric Inert Gas Counterdiffusion was selected in contrast with intravenous gas infusion as the method for inducing venous gas embolism of the lung in the present project because:

Gas emboli are generated in peripheral tissues, as they are in decompression sickness. Occurrence of local tissue damage, patterns of "Bubble" size, pathways of transit to lung, and contact with blood, are all relevant to hypobaric decompression phenomena.

Formation of gas emboli is spontaneous, from supersaturation tissue or blood.

Degree of venous gas embolism is controllable and stable, aiding dose-effect study.

Duration is prolonged, aiding duration-effect study.

Stable VGE rate is adaptable to study of gas lesion therapy (reversal).

3.0 PROJECT GOALS AND SCOPE

3.1 Initial Plan - For Sequential Program:

The planned goals of the overall program included:

(a) Primary determinations of the pathophysiologic character and development rate of pulmonary tissue damage and transpulmonary gas embolism induced by counterdiffusion venous gas embolism.

(b) Determination of effects of prior acute pulmonary oxygen poisoning on VGE-induced pulmonary pathology.

(c) Determination of volume and mass rates of VGE induced by isobaric counterdiffusion in hypobaric states, for comparison with normobaric and hyperbaric states.

The expanded scope, specific findings and duration of the primary (pathophysiologic) investigations made it desirable and necessary to place entire emphasis upon this cited Primary Goal (and its hypothesis that "Progressive Microvascular and Other Pulmonary Damage Will Result From Continuous Isobaric Supersaturation Venous Gas Embolism").

The stated ancillary goals, each with a scope larger than the examination of VGE effects on normal lung, will require specific additional investment.

3.2 Scope of Report

This Report provides (a) results of interrelated investigations of cardiorespiratory, hematologic and pulmonary effects of continuous counterdiffusion gas embolism, and (b) contrast of pulmonary histopathologic effects of isobaric counterdiffusion and air injection venous gas embolism.

4.0 METHODS OF VGE EXPOSURE AND MEASUREMENTS

Details of isobaric exposure and control conditions, monitoring and specific measurement methods are defined in the expanded description of Appendices A, B and C. Most physiologic measurements were common to each major series of experiments.

4.1 Isobaric Counterdiffusion VGE Generation

Young Yorkshire Piglets, anesthetized with I.P. Na pentobarbital, were artificially ventilated at stable level for one to two hours with a near-normoxic N₂O-O₂ mixture in an air environment. While continuing N₂O-O₂ a large or smaller area of the body surface was enveloped by a plastic bag and exposed to helium flow at 1.0 ATA. Initiation of VGE was detectable at an average of about 80 minutes from the beginning of helium exposure, as monitored by a doppler bubble detector cuff about the inferior vena cava. VGE continued until death of the animal or other termination of the experiment (Appendix B).

4.2 Monitoring and Measurements for Cardiovascular and Respiratory Effects.

Volume-controlled artificial respiration was maintained via surgically implanted tracheal cannula, at a tidal volume and respiratory rate providing an initial 36 to 40 mm Hg end-tidal PCO₂ (Appendix B). Indwelling catheters were used for blood pressure monitoring, fluid infusion, and blood sampling. A Swan Ganz catheter inserted via the right external jugular vein into a branch of the pulmonary artery provided for periodic thermodilution measurement of cardiac output, and continuous measurement of pulmonary artery pressure. Body temperature of the thermally-supported animal was continuously recorded from a rectal thermistor probe, heart rate from electrocardiograph lead II.

4.3 Sampling and Measurements of Blood Functions

Respiratory, Coagulation, Cellular Composition and other characteristics of blood were determined in different experiments series, as described in detail in Appendices B and C. Timing of blood function sampling related to the goals of specific exposure series. Common sampling and measurement procedures included:

Hematologic measurements. Blood samples obtained via external jugular venous catheter were analyzed by Automatic Cell Track 560 for hemoglobin concentration, hematocrit, white cell total and differential counts and platelet counts. Counts were normalized to hemoglobin concentration.

Blood oxygen and carbon dioxide partial Arterial pressures and pH were measured on heparinized samples, using Corning 165/2 electrodes, Radiometer Precision Buffers and Laboratory validated O₂/CO₂ gas mixtures. Values were corrected to measured rectal temperature.

Coagulation Factors were measured as described in Appendix C, on venous blood samples collected in sodium citrate with e-aminocaproic acid.

4.4 Pulmonary Microcirculation - Serial Biopsy of Lung Tissue During Continuous Venous Gas Embolism

Biopsies of the right lung were taken at 0, 2 and 6 hours of stable venous gas embolism, through two right-sided thoracotomy incisions (10th, 6th interspace), made and sealed during initial experiment preparation. The left lung remained undisturbed, for use in post-experiment necropsy studies. Biopsies and necropsy lung samples were processed for Light and Electron Microscopy histologic examinations (Appendix D).

5.0 PATHOPHYSIOLOGIC EFFECTS OF VGE

Effects of VGE on cardiovascular/respiratory functions, blood functions and pulmonary microstructure are summarized separately here, and elaborated in Appendices.

5.1 Whole Trunk and Extremities, Lethal Counterdiffusion (Appendix B).

Counterdiffusion exposure of full trunk and extremities was used to investigate cardio-respiratory pathophysiologic changes leading to death. Initial experiments, limiting counterdiffusion to hindquarters, generated cutaneous gas lesions but did not produce definitive and progressive changes in measured cardio-respiratory indices up to 18 hours of N₂O/He/1 ATA exposure.

Venous gas embolism did not occur prior to visible cutaneous changes associated with development of gas lesions. Vena Caval Doppler signals of gas embolism began at an average lag of 1.5 hrs. from the initiation of counterdiffusion. Cutaneous lesions and VGE signals continued through the over ten hours average time (range 5.4 to 28.0 hours) of survival.

Patent atrial septal defect. Special importance is given to one animal, which died abruptly after only 30 minutes of N₂O/He counterdiffusion. Necropsy showed a patent atrial septal defect, and the animal was not included in the primary series of 8 normal animals.

Cardiovascular effects. No significant cardiovascular changes occurred until onset of VGE, when pulmonary artery pressure increased from a control value of about 17 mm. Hg to about 28 (Figs. 1 and 2, Table 1). It remained elevated throughout counterdiffusion, until the circulatory failure related to death. Pulmonary capillary wedge pressure, indicative of left atrial pressure, remained at or below normal. Heart rate rose progressively, with decrease in cardiac output. Rise in hematocrit and hemoglobin concentration indicated hemoconcentration and decrease of plasma volume (Figs. 3 and 4). Most animals developed a systemic arterial hypotension 1 to 2 hours prior to death.

The composite effect appeared to be that of peripheral circulatory damage, with loss of circulating blood volume (2), decrease in cardiac output, and peripheral hypoxia, paralleled by decrease in pulmonary-arterial gas exchange, with resultant arterial hypoxia and hypercapnia (Table 1). Because artificial ventilation was maintained, final failure is considered to have been cardiovascular. Without the continuous artificial ventilation, which was a necessary condition of the several experiment series, death would have occurred within one to two hours, as in earlier investigations of the counterdiffusion mechanism (12)(19).

Arterial Blood PO₂, PCO₂ and pH. In spite of constant volume rate of artificial respiration and periodic pulmonary inflation to counteratelectasis, counterdiffusion induced progressive arterial hypoxia, hypercapnia and increased hydrogen ion concentration (Table 1, Figs. 7 and 8). These changes were evident at one and four hours of gas embolism, indicating development of hypoxic lung units. The composite effect on arterial blood represented a combined "metabolic" and "respiratory" acidosis.

5.2 Effects on Blood Components and Systems (Appendix C).

Effects of continuous venous gas embolism on blood were determined during the initial and early stages (one to four hours) of the active counterdiffusion process. Venous blood was sampled for measurements of cells, hemoglobin and functional activity of contact factors. Values of white cell and platelet counts (Figs. 5 and 6, Table 1) were normalized to baseline hemoglobin concentration to take change in plasma volume into account. Observations relevant to VGE and other gas lesion development included:

Hemoglobin concentration and Hematocrit increased at one and four hours.

Plasma volume change (Calculated from increased Hb and hematocrit) was estimated to have been reduced 15% at one hour and 26% at 4 hours of VGE.

White cell and platelet counts did not change significantly from baseline levels for all animals at one hour of exposure. However platelet counts were clearly decreased in longer exposures. The platelet aggregation phenomena described elsewhere could not be studied in this project (17).

Significant changes related to blood contact system or coagulation did not occur in normalized values for a PTT, PT, Fibrinogen levels or functional activity of Factors XII, XI, PK and Hmwk at 1, 4 and 6 hours of counterdiffusion (Table 1). The effects of low level superficial isobaric counterdiffusion with venous gas embolism may differ at least in degree from severe, generalized decompression sickness.

5.3 Effects on Lung and Pulmonary Microstructure

Studies performed relative to effects of VGE upon lung were made for primary isobaric counterdiffusion information and for comparison with the several other investigations involving pulmonary effects of controlled intra-atrial air infusion (1)(5)(10)(13)(16)(18)(21)(23). The present program included pulmonary hemodynamics, circulating leukocytes, pulmonary edema, broncho-alveolar lavage for cells and protein, and morphologic studies of gross lung, pulmonary microstructures, and ultrastructure of pulmonary capillary endothelium. Findings included:

Rise in pulmonary arterial pressure occurred at onset of VGE. No indication was found that this prompt rise is not primarily caused by physical obstruction of pulmonary arterioles and capillaries.

Pulmonary edema development was not evident from gross or microscopic examination. Lung "wet to dry weight ratio" at death was not different from control, unembolized animals.

Broncho-alveolar lavage performed sequentially after one and four hours of counterdiffusion venous gas embolism showed no significant changes in protein content or number or types of cells.

Light microscopic examination showed preservation of normal microstructure, with no evidence of capillary blockage by gas "bubbles".

Systematic electronmicroscopic examination of sequential biopsy and post-mortem lung tissue showed no evident pulmonary histopathology, leukocyte or platelet aggregations, or trapped gas emboli in exposures to prolonged, spontaneous counterdiffusion venous gas embolization of the lungs. This is in contrast to prior observations with intra-atrial air infusion in sheep and dogs (Table 2) (1)(16)(Appendix D).

Air bubble infusion into the right atrium of pigs, at the size and rate found in sheep to develop rise in pulmonary artery pressure used in the continuous lower body counterdiffusion studies in pigs (1), produced much more severe pathophysiologic effects in the pig than when used in sheep (i.e. gross elevation of pulmonary arterial pressure, hypoxemia, and passage of gas bubbles through the lungs into the arterial bloodstream, in the absence of cardiac septal defect). Air infusion has been now widely used in several different mammals (rabbit, dog, sheep, pig) for study of effects on transpulmonary passage of gas emboli and microvascular structure (1)(3)(5)(6).

Use of air bubble infusion in the pig at a rate about one tenth that customary for pulmonary embolism experiments in sheep produced the stable, 300 to 500% increase of pulmonary arterial pressure intentionally generated for the counterdiffusion venous gas embolism studies. With this lower rate of air infusion the histopathologic effects on pig lung microcirculation resembled those seen in sheep infused with the larger rate of air infusion required to produce the equivalent sustained increase in pulmonary artery pressure.

6.0 SUMMARY INTERPRETATIONS

Correlation of results from the several series of venous gas embolism experiments has provided a quantitative pattern of early pathophysiologic effects, their progression, and events preceding death. Lung biopsy and necropsy findings provide visible indexes of the presence or absence of lung microvascular microstructural change associated with continuous gas embolism of extreme degree and duration. Conclusions from these measurements and visual observations, to be elaborated in detailed open publications, include:

6.1 Survival

When the entire area of trunk and extremities (excluding only head and neck) is exposed to N₂O/He/1 ATA counterdiffusion, a near maximum degree of spontaneous, peripherally generated one ATA counterdiffusion venous gas embolism, pulmonary embolic bombardment, and bubble-venous blood interaction should be generated. In this situation, cumulative effects and pathophysiologic changes are not all clearly evident early (e.g., one hour), but become larger and even lethal in prolonged, continuous exposure.

Survival during such near-maximum exposure can approach ten hours. Survival during small degrees of continuous venous gas embolism can probably

be considered practically unlimited in normal animals. Survival in the presence of patent atrial septal defect can be brief, with sudden death early in counterdiffusion. Any degree of detectable arterial gas embolism must be considered serious (9).

Approximately 30% of animals exposed to entire trunk/extremity counterdiffusion until death demonstrated visible left-sided gas embolism at gross necropsy.

6.2 Development of cutaneous gas lesions and venous gas emboli.

Doppler indication of initiation of VGE in every instance followed development of skin lesions. Lag in development both of skin lesions and VGE was unrelated to area of superficial counterdiffusion. The degree of venous gas embolism is known to be related to the area of counterdiffusion, the gas species, and partial pressure gradients (14).

6.3 Cardiovascular Effects

Continuous venous gas embolism generated a rise in pulmonary artery pressure, proportional to surface area of counterdiffusion and persistent until ultimate systemic circulatory failure.

Systemic arterial blood pressure was initially sustained by increase in peripheral vascular resistance, even with progressive decrease in cardiac output. Pulmonary venous pressure (pulmonary capillary wedge pressure) remained normal until terminal cardiac failure.

6.4 Blood Cellular Composition

The gradual increases in hematocrit and hemoglobin concentration indicate a decrease in plasma volume. In the absence of pulmonary edema this was most probably related to the extensive tissue damage at sites of superficial gas lesion development.

While total white cell concentration did not change, the proportion of neutrophils did increase. The basis and importance of this is not known.

Thrombocytopenia may have begun early in counterdiffusion gas embolism, but it became significant in degree only late in the prolonged gas embolism experiment exposures.

6.5 Lung microstructure, cells and extravascular water.

The principal conclusion is that prolonged sub-lethal degree of isobaric counterdiffusion VGE (e.g., lower half of body) does not cause microembolic injury to the lungs, compared with the prominent defects produced by direct air infusion into mixed venous blood.

Counterdiffusion venous gas embolization of the lungs (lower half of body only), even after causing four hours of sustained pulmonary arterial hypertension, produced no visible intra-capillary trapped gas emboli, or gross

or interstitial edema, or micro-damage to alveolar capillary endothelium. Each of these pathogenic events occurs on right atrial infusion of air into air breathing sheep (1). The implication is that major pathophysiologic effects of counterdiffusion gas lesion disease, like initial degrees of decompression gas lesion disease, are related to peripheral and systemic (extra pulmonary) sites.

An explanation is not now obvious for this important difference in pulmonary effects between air embolus infusion during air breathing and N₂O/He counterdiffusion gas embolism, especially in the face of the experiment conditions of imposed equivalent influence upon degree of induced pulmonary artery hypertension.

Considerations of differences in addition to composition include the size ranges of venous gas emboli, the volume rate of gas emboli of different size range, and dynamic persistence of individual gas emboli in the hundreds of thousands of units of lung microvascular targets.

"Persistence duration" for trapped emboli is related to the gas pressure gradient between gas embolus and adjacent alveolar gas space, and to diffusivity characteristics of gases involved (20) (primarily nitrogen, nitrous oxide and helium, since O₂ and CO₂ pressures should be only little different in the two forms of experimental gas embolism). It has been shown that rate of intra-pulmonary resolution of air microemboli is affected by respiration of other gases (20). In the present project the respiration of gas mixtures were stable, and the approximate composition of the venous gas emboli was known from prior experiments (14).

In air infusion into an air breathing, nitrogen saturated animal, the nitrogen partial pressures in venous blood, venous gas emboli and alveolar gas space will not differ greatly (20). Therefore rates of resolution of gas emboli trapped in pulmonary microvessels should be slower for air infusion than for emboli containing inert gas species other than those inspired. The experimental condition of N₂O/He/1 ATA isobaric superficial counterdiffusion involves: prolonged (near equilibrium) respiration of N₂O-O₂, residual levels of nitrogen in all tissues including venous blood, and entry of helium into cutaneous venous blood by diffusion.

Venous gas emboli to the lung in the counterdiffusion state therefore contain N₂O, N₂ and He as well as O₂, CO₂ and H₂O (14). Inspired gas ventilating the alveoli contains only N₂O as the oxygen vehicle. The essentially full outward gradient for the gas embolus nitrogen and helium should initiate and accelerate an embolus collapse process, toward complete resolution or toward prompt passage of a resulting smaller microbubble through the pulmonary arteriole and capillary, to the arterial bloodstream.

Such an effect would be even more accelerated in the situation of oxygen breathing during or after venous gas embolism generated by any means, including hypobaric decompression or decompression from hyperbaric states. Size range of spontaneous micro-gas emboli would also be important to duration of entrapment in these forms of decompression venous gas embolization.

6.6 Respiratory Gas Exchange

Under constant volume pulmonary ventilation, intended to maintain an experiment condition of stable arterial PO_2 and PCO_2 , venous gas embolism caused a progressive rise in arterial PCO_2 , fall in arterial PO_2 , and increase in arterial hydrogen concentration. In the absence of evident pulmonary edema or histologic signs of lung damage, the observed changes in arterial PO_2 and PCO_2 (Figures 7 and 8) are more likely due to ventilation/perfusion mismatch than to lung microstructural injury or acute microinflammatory responses.

6.7 Transpulmonary Gas Embolism

It is probable that three extremes of transpulmonary gas bubble passage occurred in the continuous counterdiffusion venous gas embolism exposures of this project.

One was the early and promptly fatal passage of gas emboli through an identified inter-atrial cardiac septal defect, into the arterial bloodstream.

The second observation was the occurrence of grossly visible arterial gas emboli generated only in the final stages of survival, after hours of an essentially constant degree of counterdiffusion venous gas embolism to the lungs. This resembles the now recognized occurrence of visible and Doppler detectable transpulmonary embolism at large rates of intra-atrial air injection, overloading a pulmonary "filtering" capability (3)(1)(21). Visible arterial gas emboli, with inevitable access to all tissues, were seen in coronary and other arteries in the gross necropsy examinations of these experiments. Effects on organ function of such visible arterial emboli have to be prominent or fatal.

The third kind of necropsy observation, made also in many previous $\text{N}_2\text{O}/\text{He}/1$ ATA counterdiffusion studies (14), was the occurrence of gas "bubbles" in the venous blood of individual organs such as kidney, heart or intestine. Since deep organs are not subjected to superficial counterdiffusion this occurrence is considered as indicating passage of micro-emboli through the lungs into arterial blood, and then to and through all organs and tissues, with possible recirculation until resolution or growth. An ancillary probability is that such diffusely distributed micro-emboli may grow in size after death or in any decreased tissue blood flow, where tissue and organ venous blood are for any reason supersaturated (as in deep tissue counterdiffusion or decompression states). Expansion after death may represent a unique expression of a magnification of the size of gas emboli which would in life be again presented to the lung for filtering. The relation of nitrous oxide breathing to this post-mortem growth of "arterial or deep tissue gas bubbles" is probably highly important.

7.0 REFERENCES

References most relevant to this Summary Report are cited below. More extensive references to venous gas embolism and its effects are cited in draft documents of Appendices A, B, and C.

1. Albertine, K.H., J.P. Wiener-Kronish, K. Koike and N.C. Staub. Quantification of damage by air emboli to lung microvessels in anesthetized sheep. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57(5):1360-1368, 1984.
2. Brunner, F.P., P.G. Frick and A.A. Buhlmann. Post decompression shock due to extravasation of plasma. Lancet 1:1071-1073, 1964.
3. Butler, B.D. and B.A. Hills. Transpulmonary passage of venous air emboli. J. Appl. Physiol. 59(2):543-547, 1985.
4. Butler, B.D. Biophysical aspects of gas bubbles in blood. Med. Instrum. 19:59-62, 1985.
5. Butler, B.D and B.A. Hills. The lung as a filter for microbubbles. J. Appl. Physiol. 47(3): 537-543, 1979.
6. Butler, B.D. and B.A. Hills. Effect of excessive oxygen upon the capability of the lungs to filter gas emboli. In: Underwater Physiology VII, 95-102, edited by A.J. Bachrach and M.M. Matzen, Undersea Med. Soc.: Bethesda, MD, 1981.
7. Graves, D.J., J. Idicula, C.J. Lambertsen and J.A. Quinn. Bubble formation in physical and biological systems: A manifestation of counterdiffusion in composite media. Science 179: 582-584, 1973.
8. Harvey, C.A. Shallow saturation hyperbaric exposures to nitrogen-oxygen environments and isobaric switches to helium-oxygen. Undersea Biomed. Res. 4: A15, 1977.
9. Hjelle, J.O. Arterial bubbles during decompression in a deep saturation dive. Undersea Biomedical Research, (suppl.) vol. 14, No. 2, March 1987.
10. Hlastala, M.P., H.T. Robertson and B.K. Ross. Gas exchange abnormalities produced by venous gas emboli. Respir. Physiol. 36(1): 1-17, 1979.
11. Horrigan, D.J.Jr., J.M. Waligora, J. Gilbert, B. Edwards, J. Conkin, and J. Stanford. Decompression in Space. In: The Physiological Basis of Decompression Sickness, R.D. Vann, Ed. Undersea and Hyperbaric Medical Society Publication No. 75 (Phys) 6/1/89. Bethesda, MD, 1989.

12. Idicula, J., D.J. Graves, J.A. Quinn and C.J. Lambertsen. Bubble formation resulting from the steady counterdiffusion of two inert gases. In: Underwater Physiology V. Lambertsen, C.J. (ed.) Bethesda, MD: FASEB, 1976. pp. 335-340.
13. Johannes, M., A. Tucker, E.K. Weir, J.T. Reeves and R.F. Grover. Platelet-mediated pulmonary hypertension and hypoxia during pulmonary microembolism. Chest, 74:6, 1978.
14. Lambertsen, C.J. Relations of isobaric gas counterdiffusion and decompression gas lesion diseases. In: Vann, R.D., Ed. The Physiological Basis of Decompression: UHMS Publ. 75 (Phys) Bethesda, MD: Undersea and Hyperbaric Med. Soc., 87-106, June 1989.
15. Lambertsen, C.J. and J. Idicula. A new gas lesion syndrome in man induced by "isobaric gas counterdiffusion". J. Appl. Physiol. 39: 434-443, 1975.
16. Moosavi, H., H.J. Utell, R.W. Hyde, P.J. Fahey, B.T. Peterson, J. Donnelly and K.D. Senzen. Lung ultrastructure in noncardiogenic pulmonary edema induced by air embolization in dogs. Lab Invest. 45(5): 456-464, 1981.
17. Neuman, T.S., R.G. Spragg and H. Wohl. Platelet aggregates following decompression. Undersea Biomed. Res. 8 (suppl.), 42, 1981.
18. Okuda, K., K. Nakahara, A. Binder, and C. Staub. Venous air emboli in sheep: reversible increase in lung microvascular permeability. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51, 887-894, 1981.
19. Pisarello, M. Fried, D. Fisher and C.J. Lambertsen. Superficial isobaric counterdiffusion gas lesion disease - effects leading to mortality. In: Proceedings of the Eighth Symposium on Underwater Physiology. Bethesda: Undersea Medical Society, 101-106, 1984.
20. Presson, R.G., K.R. Kirk, K.A. Haselby, and W.W. Wagner, Jr. Effect of ventilation with soluble and diffusible gases on the size of air emboli. J. Appl. Physiol. 70(3):1068-1074, 1991.
21. Vik, A., A.O. Brubakk, T.R. Hennessey, B.M. Jenssen, M. Ekker, and S.A. Slondahl. Venous air embolism in swine: transport of gas bubbles through the pulmonary circulation. J. Appl. Physiol. 69(1):237-244, 1990.
22. Waligora, J.M., D.J. Horrigan, A.T. Hadley III and J. Conkin. Verification of an altitude decompression protocol for shuttle operations, utilizing a 10.2 p.s.i. pressure stage. NASA Technical Memorandum 58259, Johnson Space Center, Houston, TX, June 1984.

23. Yoshida, M., I. Sekalay, H.H. Rotkman and P. Kimbal.
Pulmonary vascular changes following air embolism in the dog.
Archives Internationales de Physiologie et de Biochime, 86, 317-
325, 1978.

8.0 FIGURES and TABLES - Continuous Isobaric Counterdiffusion
(Individual Effects, 8 Animals)

Figure 1. BLOOD PRESSURES AND HEART RATE
(Mean Arterial BP, Mean Pulmonary Artery BP,
Mean Pulmonary Wedge Pressure)

Figure 2. CARDIAC OUTPUT

Figure 3. HEMOGLOBIN

Figure 4. HEMATOCRIT

Figure 5. TOTAL WHITE BLOOD CELLS

Figure 6. PLATELETS

Figure 7. ARTERIAL PO₂

Figure 8. ARTERIAL PCO₂

Figure 9. CONTINUOUS VENOUS GAS EMBOLISM

Table 1. Detection of Lesions in the Lung Microcirculation in
Anesthetized Pigs with Counterdiffusion and Air Infusion
Venous Gas Emboli

Note: Changes During Lethal Counterdiffusion Venous Gas Embolism.
Generated by Trunk and Limb Exposure.

Effects Are Referenced to Pre-Counterdiffusion Control State.

Plotted Values Represent Trend to last Measurement before Death

Measurements Interpolated to Common Times To aid Statistical
Correlation of Effects Relative to Exposure Duration.

FIGURE 1
BLOOD PRESSURES AND HEART RATE

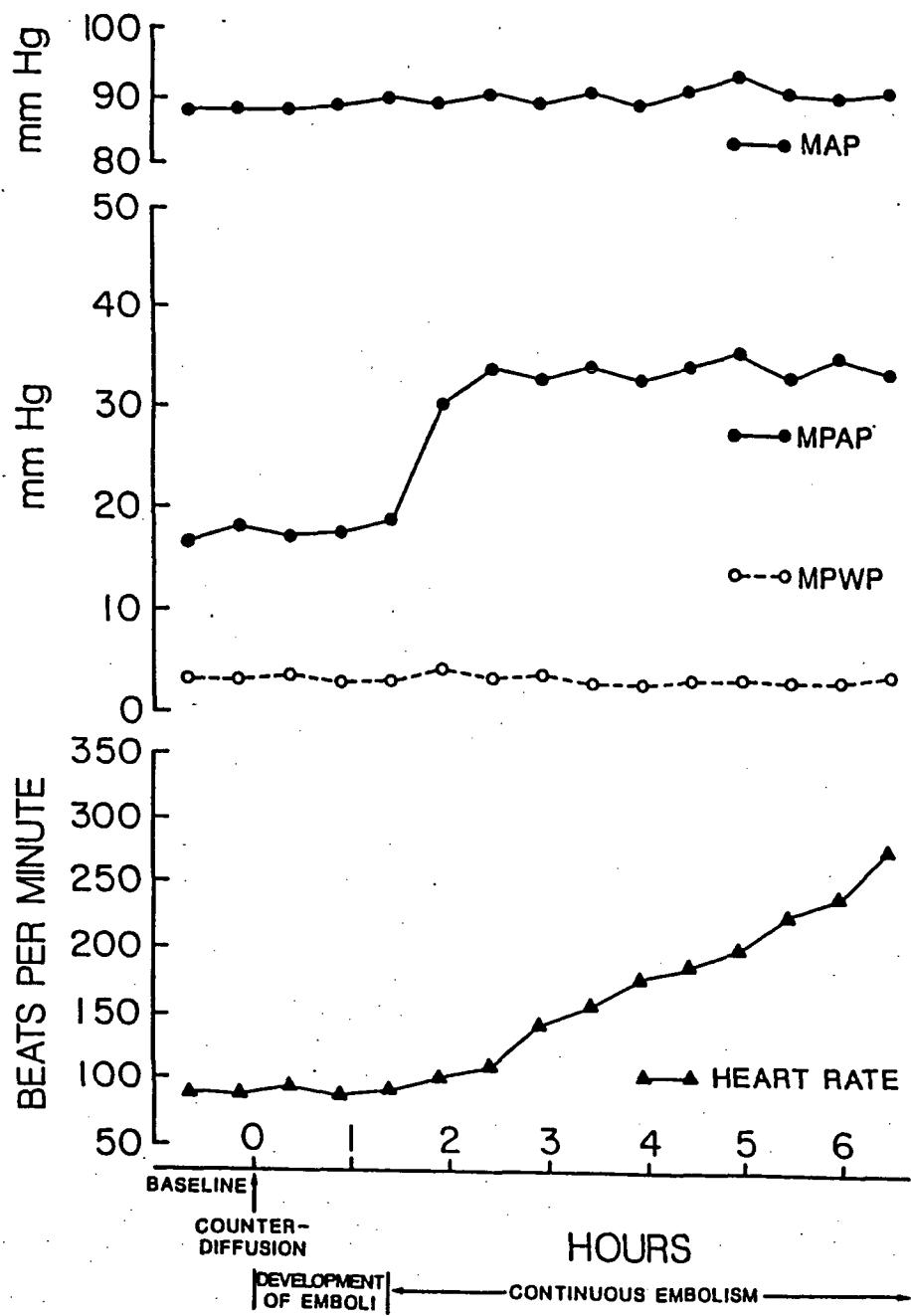


FIGURE 2
CARDIAC OUTPUT

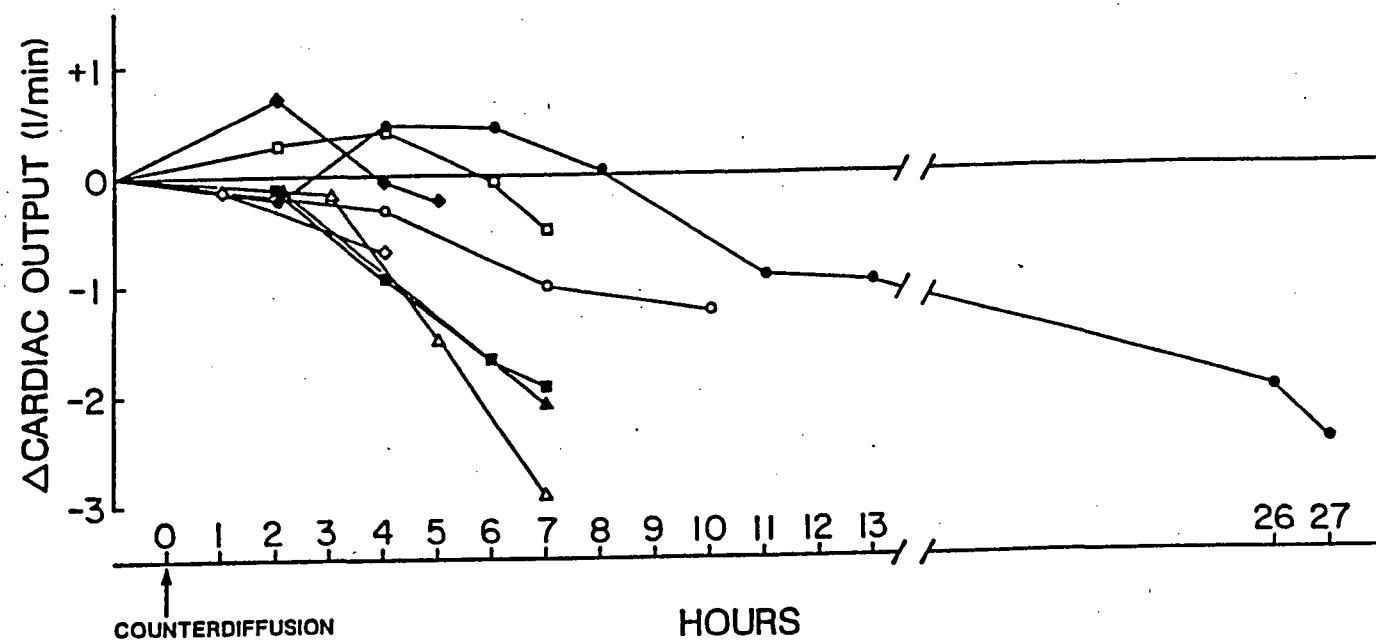


FIGURE 3
HEMOGLOBIN

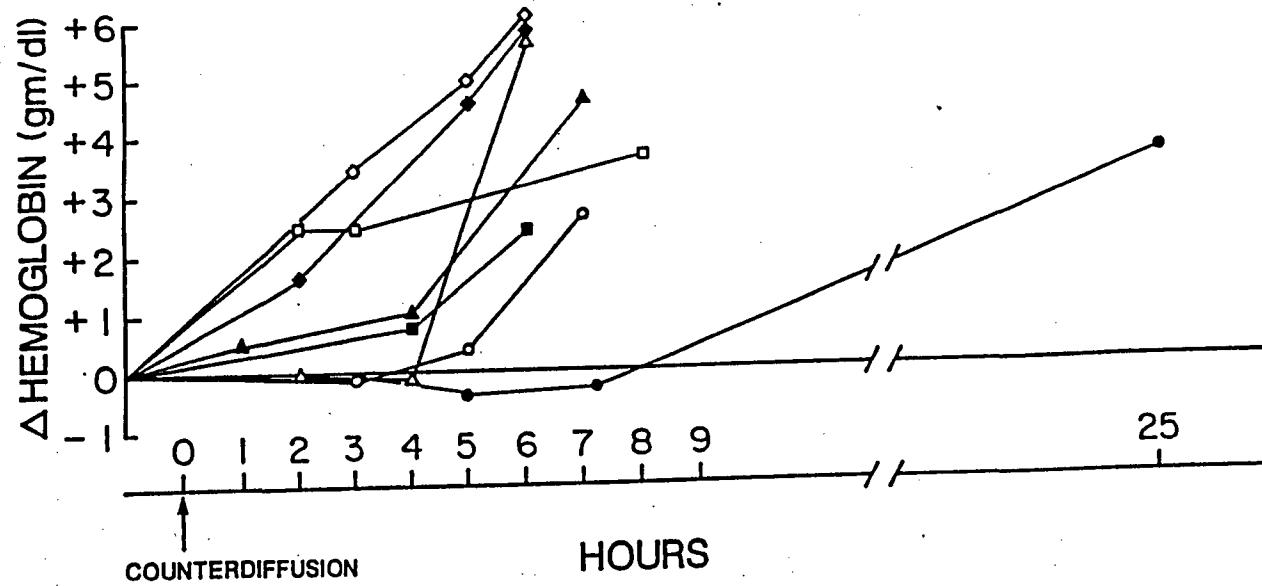


FIGURE 4
HEMATOCRIT

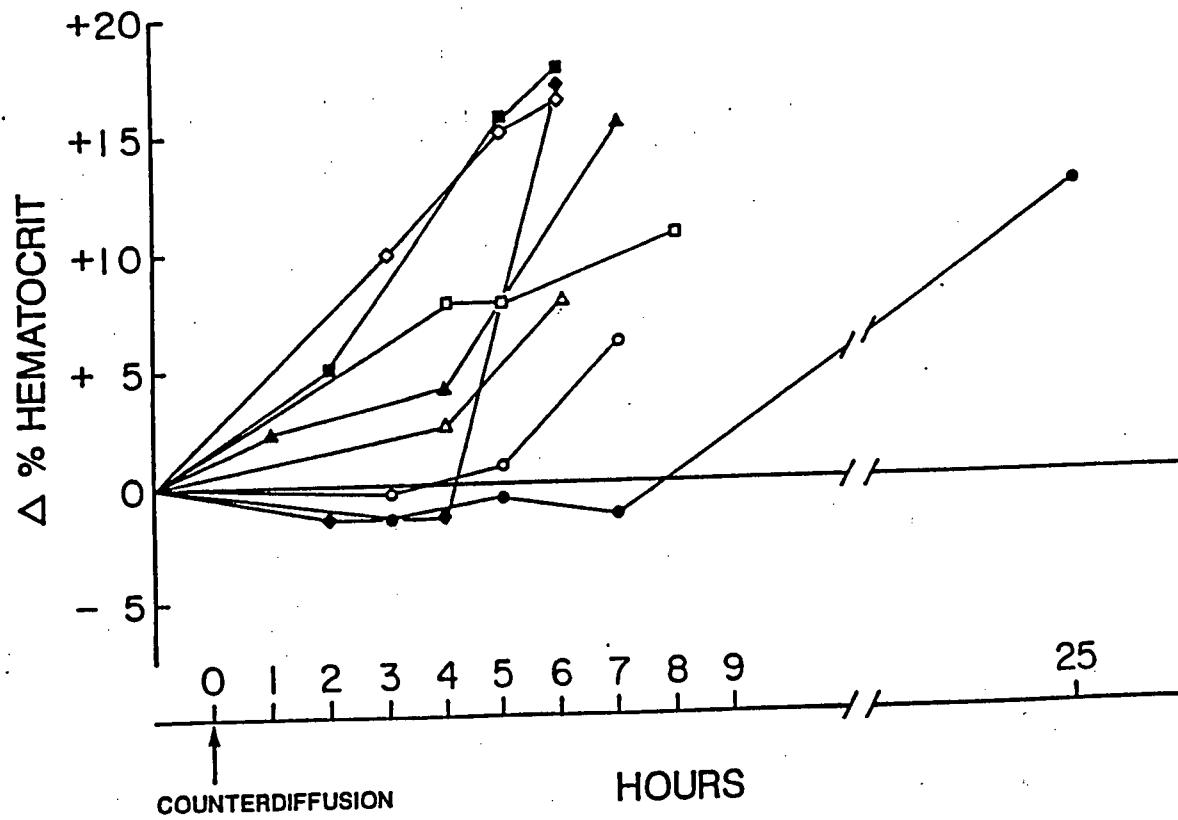


FIGURE 5
TOTAL WHITE BLOOD CELLS

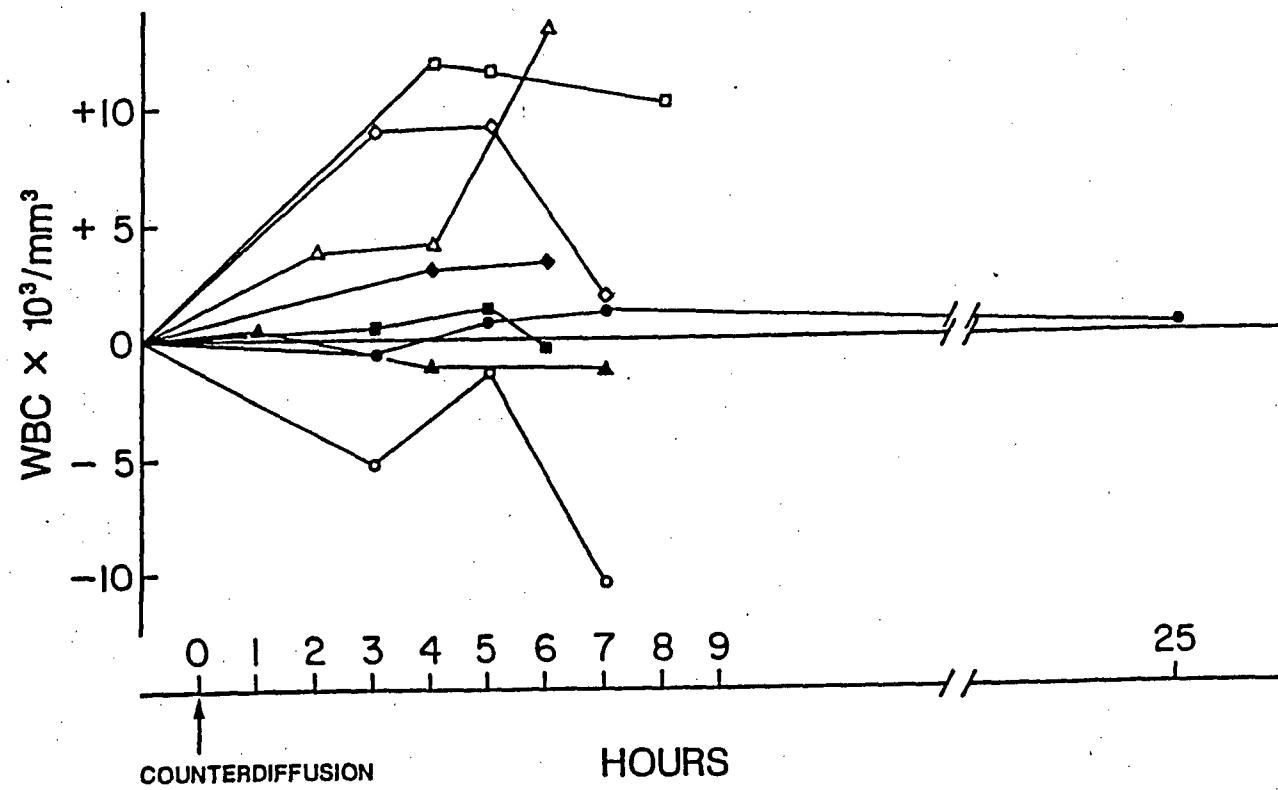


FIGURE 6
PLATELETS

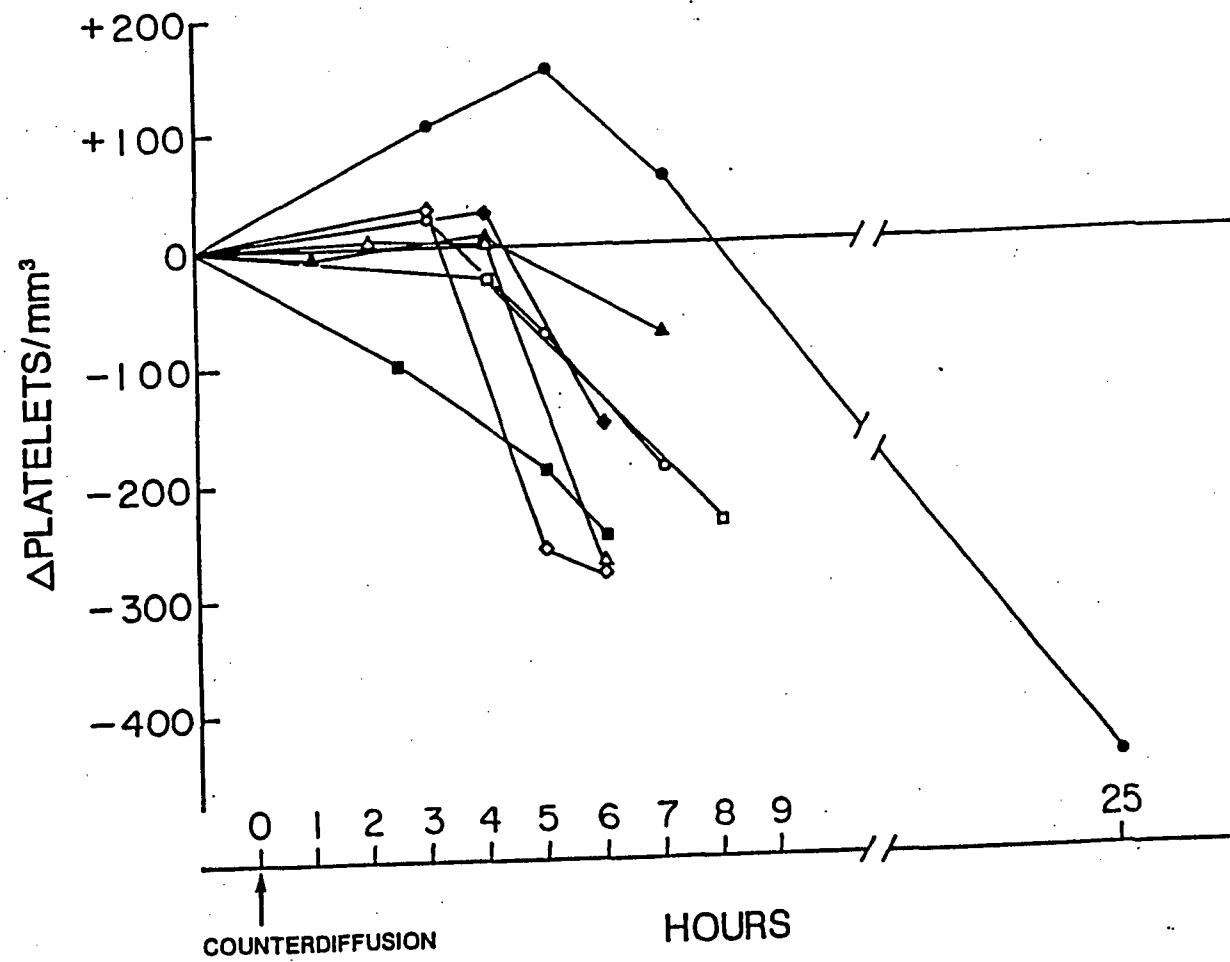


FIGURE 7
ARTERIAL PO_2

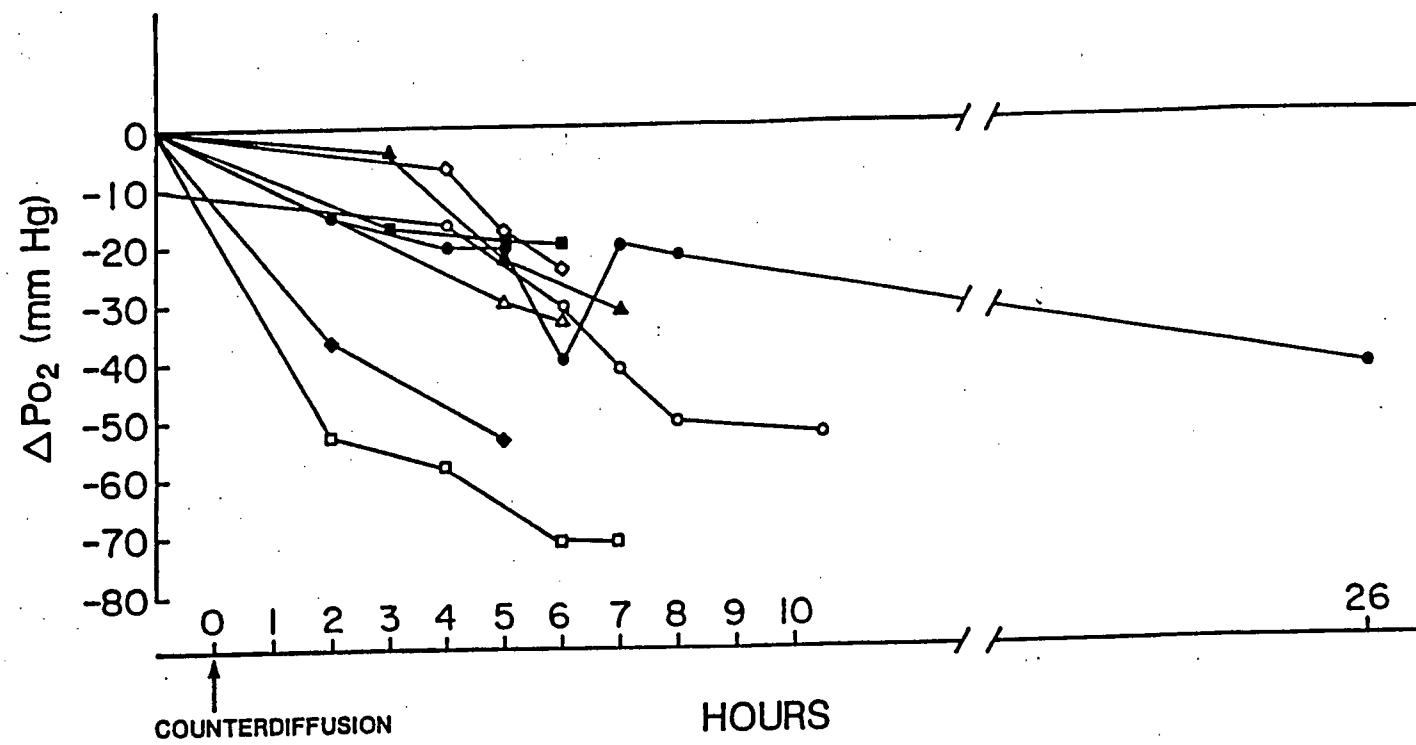


FIGURE 8
ARTERIAL PCO₂

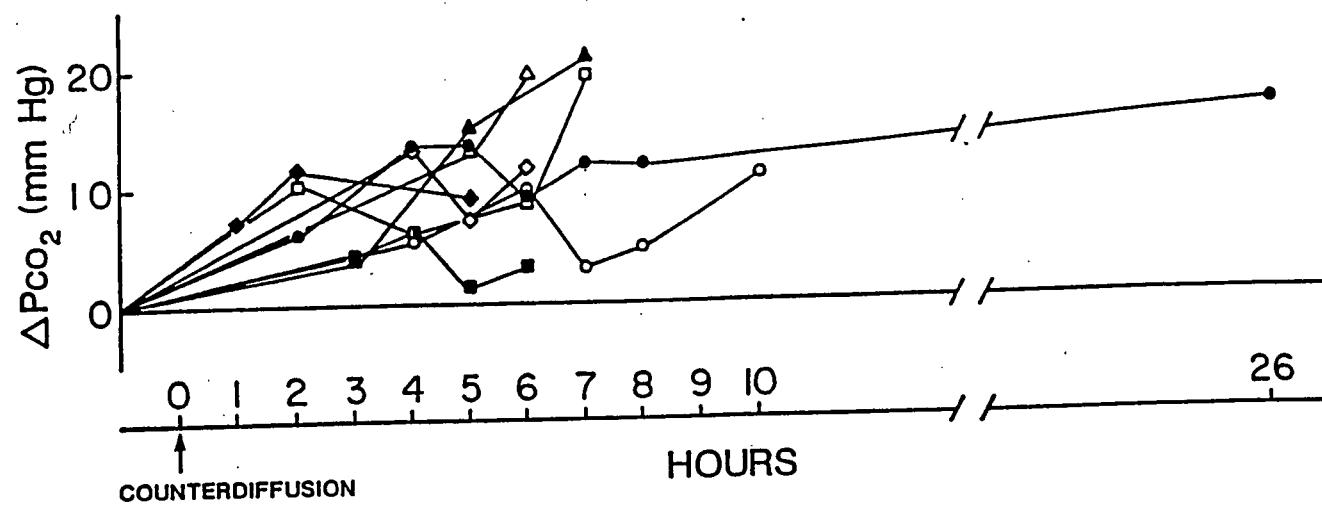


FIGURE 9
CONTINUOUS VENOUS GAS EMBOLISM
(SUPERFICIAL ISOBARIC COUNTERDIFFUSION)

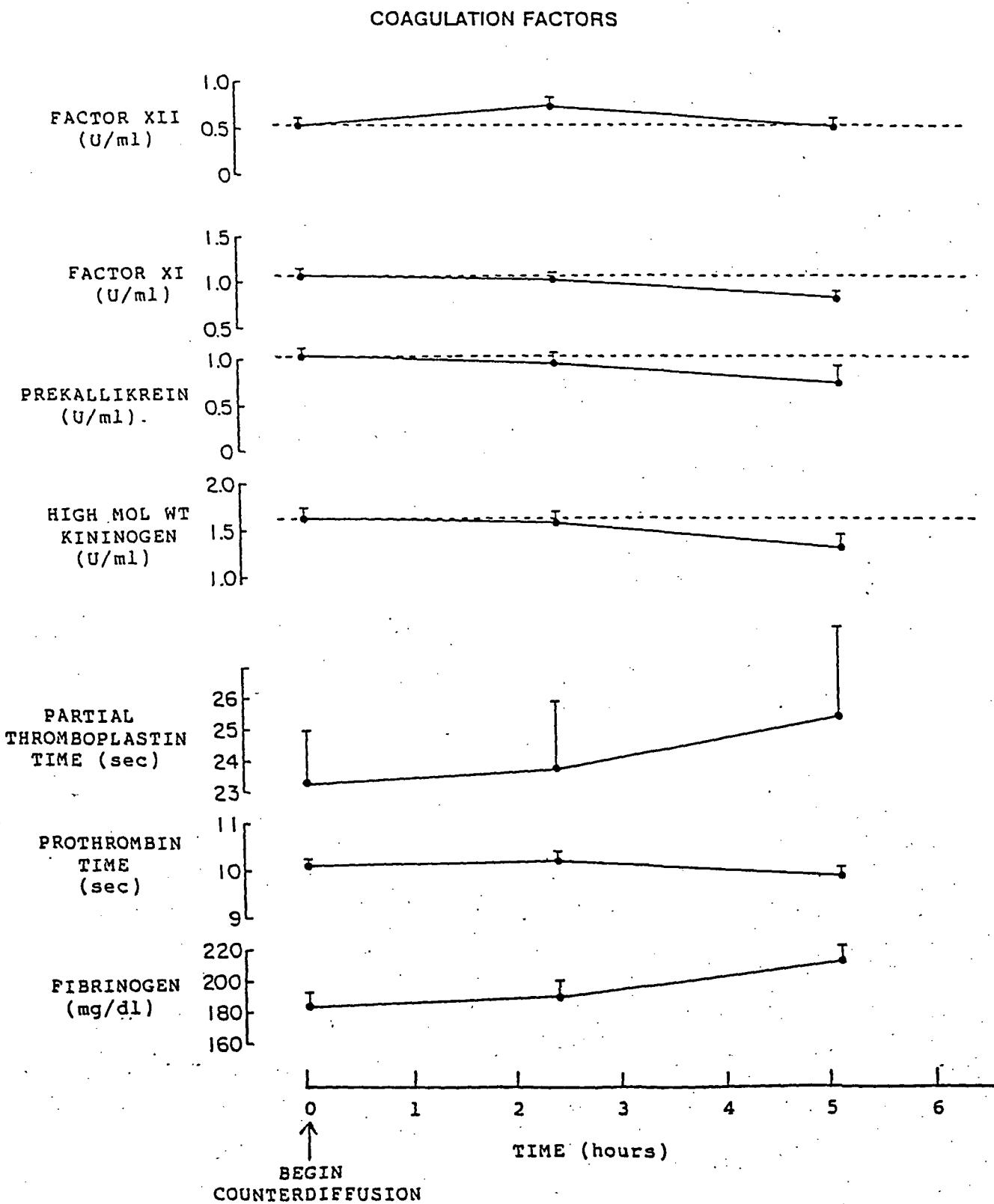


Table 1

Detection of Lesions' in the Lung Microcirculation in Anesthetized Pigs
with
Counterdiffusion and Air Infusion Venous Gas Emboli

Condition	<u>Number of Animals</u>	<u>Pulmonary Arterial Microvessels</u>		<u>Pulmonary Venous Microvessels</u>		<u>Alveolar Capillaries</u>	
		No.	Lesions	No.	Lesions	No.	Lesions
<u>Group I</u>							
Counterdiffusion	5	50	0	50	0	250	0
<u>Group II</u>							
Venous air embolization	3	50	34	50	2	150	0
<u>Group III</u>							
Control Air Breathing	5	50	0	50	0	250	0

Data are shown as the total number of vessels (No.) and endothelial lesions (gaps) within those vessels. 10 pulmonary arterial and venous vessels and 50 alveolar capillaries each were analyzed per pig in counterdiffusion and control groups. Additional arterial and venous vessels were analyzed in air infusion group.

APPENDIX A

APPENDIX A

RELATIONS OF ISOBARIC COUNTERDIFFUSION AND
DECOMPRESSION GAS LESION DISEASES

C.J. LAMBERTSEN, M.D.

Institute for Environmental Medicine
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

June 1989

From: The Physiological Basis of Decompression 38th Undersea and Hyperbaric Society Workshop. R.D. Vann, Ed. UHMS Publication No. 75 (Phys) 6/1/89.

RELATIONS OF ISOBARIC GAS COUNTERDIFFUSION AND DECOMPRESSION GAS LESION DISEASES

C.J. Lambertsen, M.D.

Institute For Environmental Medicine
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

Purpose and Perspective

A personal goal at this Conference is to use the information and concepts of isobaric counterdiffusion to bind together three key elements determining decompression safety. These elements, which cannot sensibly be separate in diving, still tend often to be considered separately. They are the oxygen effects (1), the several forms of isobaric inert gas exchanges (2) (3) (4) and the forms of decompression-induced inert gas elimination (1).

Oxygen simultaneously provides: the most predictable physical aid to degassing, a complex of physiologic forces which conceivably modify decompression-relevant de-gassing process, a component of gas spaces or emboli, and variable forms of toxic effect which conceivably can modify decompression-relevant degassing. Only the positive (the physical) role has been specifically demonstrated.

Decompression Gas Elimination would do no harm in the absence of free gas phase growth.

Isobaric Inert Gas Counterdiffusion can aid degassing or it can interfere with degassing during decompression. It can generate stable or transient gas supersaturation, gas lesions and gas emboli at stable ambient pressures, or it can generate transient subsaturation.

The relationships of oxygen, decompression and counterdiffusion are clear on conceiving a decompression or therapy as a series of instantaneous (isobaric) periods in which all ongoing forms of gas exchange and gas effect exist simultaneously (as they do in reality).

A second personal goal is again to urge use of the term "Gas Lesion Diseases," devised to encompass the several overlapping and impure pathologic states now obviously related and sometimes concurrent in exposures to unusual pressures and atmospheres (2). The still conventional blanketing designations of "Decompression Sickness" or "Gas

"Embolism" are not adequate for present and future diving or hyperbaric medicine, and have led to use of such foolish terminology as "isobaric decompression sickness."

Table 1 indicates the scope of major forms of gas lesion diseases, different in their inducing circumstances or their consequences. The resulting symptoms or objective signs (e.g. neural, vestibular, cutaneous, or local pain may be similar or different, and do not themselves describe or represent the specific disease or fundamental mechanism. Table 2 emphasizes the clearly obvious fact that the ultimate primary basis for gas phase generation in decompression or in isobaric gas lesion diseases is an excess pressure of gases in peripheral tissues including peripheral blood. The complex consequences of bubble growth and bubble-tissue interactions are sequels to the primary event, without which no pathologic effects would occur. The table also emphasizes that the very occurrence of gas lesions and emboli in isobaric states indicates the pre-existence or continuous formation of "nuclei" in normal tissue fluids. Asterisks (*) mark venous gas embolism in four of the forms of gas lesion disease to call attention to the possibility that venous gas embolic process may conceivably lead to arterial gas emboli. While venous gas emboli affect blood and lung, arterial gas embolic phenomena should be considered as having access to all tissues and arterioles. It is therefore here cited as "systemic" embolization.

Table 1.

<u>MAJOR FORMS OF GAS LESION DISEASES</u>	<u>SOURCE OF GAS PHASE</u>
Pulmonary Overexpansion Systemic Gas Embolism (Arterial) Pneumothorax Subcutaneous/Mediastinal Emphysema	Lung
Iatrogenic or Traumatic Gas Embolism Venous* Arterial Systemic (Arterial)	Extrinsic
Decompression Sickness (Post Hyperbaric, Hypobaric) Cutaneous Deep Tissue Venous Embolic* Systemic (Arterial)	Peripheral Tissue
Superficial Isobaric Counterdiffusion Sickness (Stable State) Cutaneous Vestibular Venous Embolic* Systemic (Arterial)	Peripheral Tissue
Deep Tissue Isobaric Counterdiffusion Sickness (Transient) Venous Embolic* Systemic (Arterial)	Peripheral Tissue

Isobaric Counterdiffusion and Decompression

Table 2.

GAS PHASE DEVELOPMENT IN DIVING DECOMPRESSION AND ISOBARIC COUNTERDIFFUSION

Inert gas uptake is not harmful in diving, inert gas elimination is not harmful, and decompression itself is not harmful.

Gas phase development is the pathophysiologic event, whether microscopic or gross. It is itself the result of an elevation of tissue inert gas pressure above ambient.

It is most probable that gas lesion development results from growth of normally present gas nuclei, as indicated by the isobaric development of gas lesions at one ATA, without prior compression or decompression.

Gas lesions do not produce detectable symptoms or objective signs at all target sites.

At any site, symptoms and objective signs require time to develop after gas phase development has begun.

The more conservative the limitation of degree of inert gas excess, the less severe should be the degree of symptoms or objective signs, of any type at any site.

Localization of gas phase development.

Some effects of early stages of isobaric gas phase development are precisely visible in experiment. Most of those for early stages of decompression sickness are not. Like the counterdiffusion processes, decompression sickness (excluding pulmonary barotrauma) is not a single, "yes or no" event or "threshold" phenomenon. It is potentially a generalized systemic process of gas phase separation and expansion, which may become severe enough in some microanatomical locations to be clinically diagnosed. It can probably simultaneously go unrecognized in many different other locations. Decompression sickness, like the counterdiffusion processes, is surely a diffuse continuum of graded degrees of pathophysiologic event and effect, simultaneously occurring in many scattered tissue locations, each of which has its own local "stress-effect" consequences. Categorical designations of decompression sickness effects, as for example into Type I and Type II, have been medically and operationally practical. However, they are not descriptors of the fundamental, decompression-induced systemic processes, or the manner in which they can be expected to be aggravated by forms of isobaric counterdiffusion (1) (2).

Against the license of these "Perspectives" the following review will emphasize empirical observations in experiments with isobaric counterdiffusions.

Isobaric Counterdiffusion and Decompression

Table 3.

ISOBARIC INERT GAS COUNTERDIFFUSION (Superficial and Deep Tissue Forms)

Two forms of isobaric counterdiffusion supersaturation can produce gas lesions or venous gas emboli.

"Superficial" isobaric counterdiffusion occurs through body surfaces when air or N₂-O₂ is breathed and the external environment is helium.

"Deep tissue" isobaric counterdiffusion occurs when any different inert gases are breathed in sequence.

Each form of isobaric counterdiffusion can lead to inert gas supersaturation of involved tissues. This supersaturation and gas embolus formation is now entirely preventable, by proper choice of operational gases and sequences.

Each form of isobaric generation of supersaturations can potentially exaggerate a concurrent decompression supersaturation and related gas lesions.

Each form of isobaric counterdiffusion can lead to inert gas subsaturation of involved tissues. This result is operationally and therapeutically useful.

"Isobaric" gas exchanges of all types should be considered as able to occur in the course of decompression procedures, as well as at stable pressures.

Hyperoxygenation therapy is rational for isobaric counterdiffusion gas lesions, as it is for gas lesions of decompression sickness.

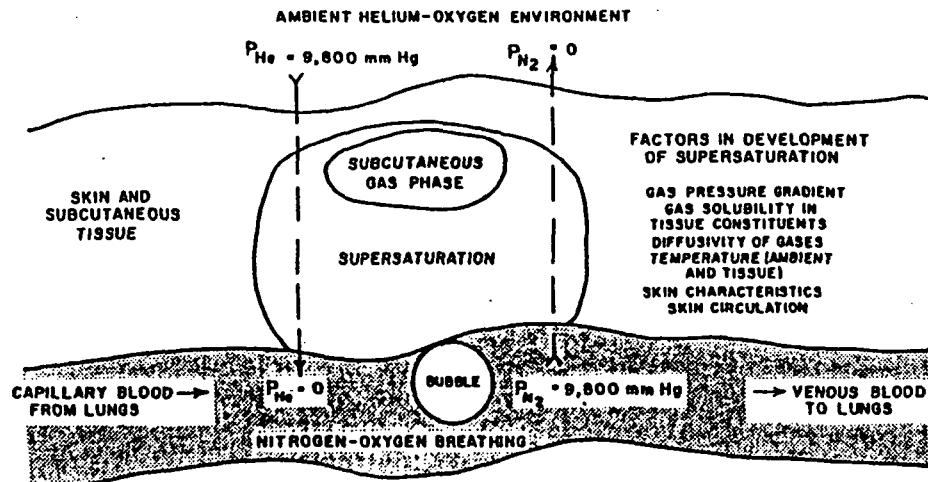


Fig. 1. Dermal Lesion in Superficial Isobaric Counterdiffusion.

Schematic of circumstances in gas bubble formation without change in ambient pressure. Excess inert gas saturation develops due to more rapid flux of one gas, e.g., helium, into the tissue and capillary as a less rapidly moving second gas, e.g., nitrogen, diffuses out to the atmosphere (4).

The Phenomenon of Isobaric Inert Gas Counterdiffusion

Counterdiffusion was first recognized and named as the cause of gas lesion development in individuals who for a broad physiologic study (3) were saturated in a helium atmosphere at constant pressure and who then breathed neon-oxygen-helium and nitrogen-helium-oxygen mixtures at pressures equivalent to 400, 700, 900, 1200 fsw (3) (4) (5). These individuals developed severe cutaneous itching, the first symptom of a

larger problem that was partially solved by encompassing them in a gas-tight suit into which they exhaled. Only regions of helium-exposed skin, the scalp, hands, and skin of the face where the mask did not cover, continued to itch (3). Cutaneous symptoms encountered in previous experiments at a lower pressure were discounted as not attributable to gas lesion development (6).

In the human subjects, continued exposure to ambient helium while breathing a slower diffusing inert gas produced hard, raised, white, bloodless, cutaneous lesions. It also produced severe vestibular dysfunction (3) (4). What did the human studies tell us about where bubbles form? Do they form in the local blood vessels or in tissue spaces (Fig. 1)? The cutaneous lesions looked as though "bubbles" had formed locally in tissue and forced out the blood. When these lesions in man were dissected with a needle at the high chamber pressures, no bleeding occurred. Venous gas embolism was not suspected.

DEPTH (ata)	EXPERIMENTAL CONDITIONS: Breathing / Chamber Gas Gas						
	Ne/He	N ₂ /He	Ar/He	N ₂ O/He	SF ₆ /He	He/N ₂ O	He/Ar
1		+	+	+	0	0	0
2		+	+		0		
3		+	+		0		
4	+	+	+		0		
7	+	+	+				
10	+	+	+				

Fig. 2. Exposures of Pigs to Superficial Isobaric Gas Counterdiffusion at Ambient Pressures From 1 to 10 ATA.

A (+) indicates that lesions were observed, an open circle (0) that no lesions were seen. The severity of lesions for any given depth and time was: N₂O > Ar > N₂ > Ne (9).

After the observations in human subjects, the cutaneous gas lesions were produced in pigs by having helium outside animals which breathed either nitrogen, argon, or neon with oxygen (Fig. 2) (19)??. Gas spaces formed not only in the most superficial layers of the skin, but also formed throughout the skin thickness, and even in subcutaneous tissue spaces. The process also reached blood vessels and caused a continuous venous gas embolism (9) (4) (2). In fact the gas spaces dissect the tissues as gas lesions expand (Fig. 3) (4) (8). The lesions generate after an initial time lag, and the process eventually leads to death from continuous venous gas embolism (2), (9). Postmortem examination shows that the vena cava is filled with gas bubbles (Fig. 4), and vessels of deep tissues such as kidney, heart and retina may unexpectedly also contain free gas (2), (9). Measurement of gas in an artificial subcutaneous depot illustrated the pattern of deep subcutaneous gas environment throughout the lethal process (Fig. 5) (23).

Isobaric Counterdiffusion and Decompression



Fig. 3. Gas spaces in section of subcutaneous tissue of pig exposed to
 $N_2O/He/1$ ATA superficial isobaric counterdiffusion (From 23).



Fig. 4. Occurrence of massive amount of gas in inferior vena cava of pig following death due to N₂O/He/1 ATA superficial isobaric counterdiffusion (2).

Isobaric Counterdiffusion and Decompression

"Superficial Isobaric Inert Gas Counterdiffusion."

The new lethal environmental hazard was christened "Superficial Isobaric Inert Gas Counterdiffusion Gas Lesion Disease." It is easily produced at one atmosphere without ever compressing or decompressing the experimental animal. However, the appropriate gases must be used. An effective combination is nitrous oxide breathing while the animal is surrounded by helium (9).

Other structures besides the skin and venous circulation are involved in isobaric counterdiffusion. Vestibular dysfunction occurred early at a stable pressure equivalent to 1200 feet of sea water in individuals breathing a neon-helium-oxygen mixture while surrounded by helium. In one, nausea, vomiting, and vertigo were so severe that he could not take even fluids by mouth for several days. After about five days, recovery allowed decompression (3).

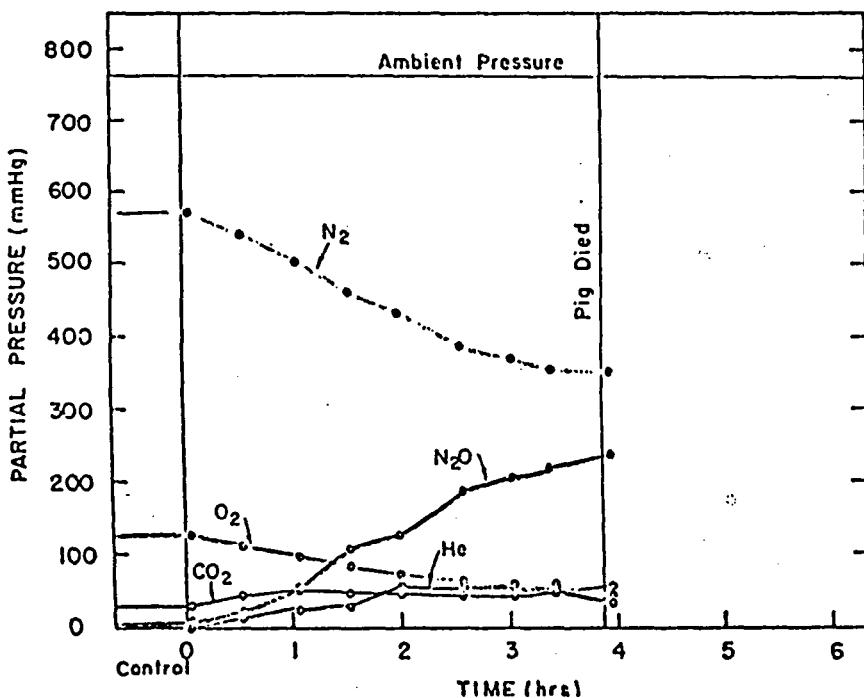


Fig. 5. Changes in Gas Partial Pressures During Superficial Isobaric Gas Counterdiffusion In Artificially Induced Subcutaneous Gas Pockets (23). (Pig Breathing N₂O-O₂, Surrounded by Helium).

What mechanism might produce such an unexpected and incapacitating "vestibular" effect at constant ambient pressure? It occurred to us that the round window membrane between the middle ear and inner ear was functionally an external surface of the body, rather than an internal structure, in spite of being located deep, internal to the thin tympanic membrane (4). Helium might therefore at the very large ambient helium partial pressure gradients experienced in 38 ATA experiments (nearly

20,000 mm. Hg.), diffuse through the tympanic membrane, then through the round window while the respired neon gas was escaping more slowly from the endolymph into the middle ear (4). Helium passage through the tympanic membrane of cats was measured, and found to be rapid (10) (11). To learn whether isobaric counterdiffusion would produce bubbles in endolymph we observed the round window by direct microscopy in guinea pigs breathing nitrous oxide at one and at two atmospheres with helium external to the round window (12). No bubbles appeared in the inner ear fluid after several hours of this exposure. The specific cause of this severe and incapacitating isobaric gas lesion in man therefore still remains an unsettled matter. It may relate to occurrence of as yet undetected arterial gas emboli.

The absence of effects of superficial isobaric counterdiffusion on the human eye is similarly unexplainable. Post-mortem photographs of the retina through the lens of the eye after prolonged counterdiffusion of pigs with nitrous oxide and helium at one ATA show gas bubbles deep, in the retinal vessels. In the human subjects who generated skin lesions there were never any symptoms, inflammation, or other evident effects of the early counterdiffusion process on vision conjunctiva, sclera, or cornea (4) (23) (13). When N₂O/He/2ata counterdiffusion was produced through the surfaces of the eye in rabbits, no bubbles or lesions had occurred by the time the exposure of the skin caused subcutaneous gas lesions, venous emboli and death (13). The eye has a surface area of the body where topical isobaric counterdiffusion occurs with no detectable adverse consequences.

We have designated the isobaric passage of gas into the body through its surfaces, and out of the body from capillaries through surfaces, "Superficial Isobaric Inert Gas Counterdiffusion" (4). Reversal of the counterdiffusing environmental and respiratory inert gases (e.g. breathing helium-oxygen while surrounded by air or argon-oxygen) showed that gas lesion formation in subcutaneous tissues and venous gas bubble emboli occur with the inward diffusion of helium and not with its outward diffusion (9) (4). The fundamental concept of superficial isobaric counterdiffusion gas phase development was analyzed mathematically and studied with in vitro models, which showed that the characteristics of gas diffusion through an aqueous/lipid interface could determine whether or not gas phase separation would occur. Different gases were made to diffuse in opposite directions through two adjacent but different materials (water and olive oil) (5). Relative diffusion rates for gases are determined by diffusivities and membrane permeabilities, which together determine whether a gas "supersaturation" would be large enough to cause bubbles to grow. Supersaturation was predicted to be maximum when the square root of the product of the diffusivities was equal to the ratio of the thicknesses of the membrane (5).

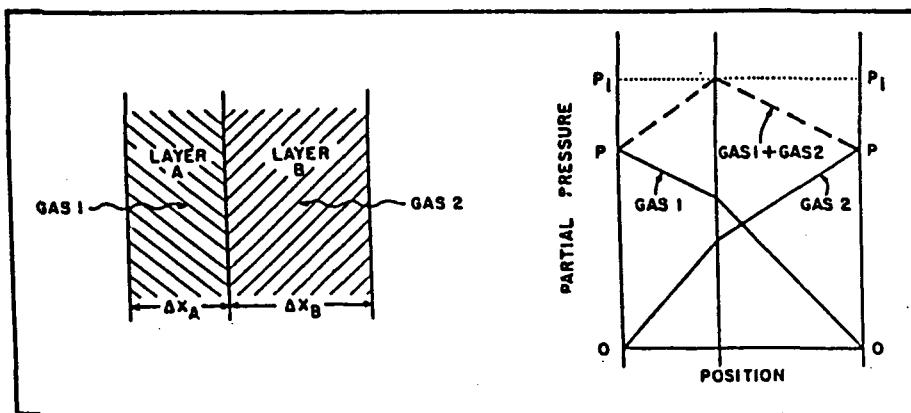


Fig. 6. Gas Partial Pressure Profiles Resulting From Steady Counterdiffusion of Two Inert Gases.

The diagram on the left indicates concept of diffusion of two different gases (1 and 2) across a dual barrier (layers A and B) having different lipid-water compositions and, hence, different permeabilities. The diagram on the right shows the gradients resulting from this counterdiffusion.

When the gases and barriers are so selected that the resistance to transport is low in the first layer encountered and high in the second, the sum of the gas partial pressures is shown mathematically to be above the ambient pressure and is at a maximum at the barrier interface (5).

Such events may be occurring in many millions of micro-loci during a human exposure.

The potential for development of a very large degree of gas supersaturation in exposed tissues is very great. At 1200 feet of seawater (or 38 ATA where the phenomenon became recognized in man), it should be theoretically possible to generate about 9 atmospheres of supersaturation if the process is allowed to proceed to its maximum extent (4) (5).

A specific and basic question is "What is the mechanism of origin of the bubbles in supersaturated tissue and capillary which lead to lethal venous gas embolism and destructive extravascular lesions at one ATA, without compression or decompression or even movement?" Without nucleation gas bubbles should not form, but bubbles do form. A necessary explanation must therefore be that there are, inevitably and continuously, pre-existing gas nuclei in normal tissue in life at one ATA. Whether nuclei persist, or randomly form and regress, or both, such nuclei would appear from the isobaric experiments to be functionally very stable, since the counterdiffusion syndrome occurs in humans even after compression to 38 ATA. These empirical observations

in animals and man must be kept part of any theoretical discussions of roles of nucleation in diving and decompression.

The Superficial Isobaric Inert Gas Counterdiffusion phenomenon deserves the designation as one of the "Gas Lesion Diseases" (4). In experiments with pigs at sea level, the process of N₂O/He/1 ATA superficial counterdiffusion could cause death within an hour and a half when the whole animal body was exposed to helium (9). To slow this rapid process

sufficiently in some experiments for detailed study, only the hind quarters of other pigs were exposed (by enclosing them within a helium-filled plastic bag). Removing the flow of gas emboli from the vena cava with a bubble trap prevented them from reaching the lungs and extended the life of the animal (14). This bubble trapping enabled measurement of the volume rate at which gas emboli were generated by counterdiffusion through a measured area of skin. It also allowed detailed study of the local lesion development, from initial to severe stages. With the animal breathing nitrous oxide and exposed to helium over the lower body and nitrogen over the upper body, the N₂O/N₂/1 ATA counterdiffusion produced no visible effect, but the N₂O/He/1 ATA counterdiffusion formed cutaneous gas lesions and venous gas emboli, disrupted skin capillaries, and had a grossly destructive effect on the skin and subcutaneous sites. A very sharp line of visible demarcation existed between the helium exposed region and the nitrogen exposed region.

Venous gas embolism does not appear at once in such experiments (2). There is a delay of approximately one half to one and one half hours before gas emboli begin to accumulate in the bubble trap in the vena cava, after which the rate of gas embolism becomes a stable process (2). During this delay gas lesion development is occurring in the periphery. At two atmospheres pressure, the volume rate of venous gas embolization is the same as at 1 ATA (about 200ml/hr./sq.m) but the mass rate is twice as great as at one atmosphere (14). The composition of the gas emboli that collect in the vena cava bubble trap is mostly nitrous oxide, some helium, along with a little nitrogen, oxygen, carbon dioxide, and water vapor (2) (14). It is important to recognize that the gas composition of venous emboli can not be considered to reflect the process of counterdiffusion at the skin surface, since the venous blood comes from many sources, superficial and deep.

This process of N₂O/He/1 ATA Superficial Counterdiffusion can be used as a controllable experimental tool to study early development of peripheral gas lesions and the progression of any pathophysiologic effects of gas emboli in the venous blood and "bombarding" the lungs.

The magnitude of effects on blood and lung can be experimentally controlled by changing the skin surface area which is subjected to counterdiffusion, or by changing the gas gradients, or by changing ambient pressure. If the area is small enough, there will be no increase in pulmonary artery pressure even though venous bubbles occur. With larger skin areas the gas embolism causes a rise in pulmonary

arterial pressure. In pigs which are monitored by venous and arterial Doppler probes, no bubbles appear to cross the lungs despite constant and continuous bombardment over six to eight hours. However, in one animal with a patent septal defect, death occurred early and gas bubbles were present in grossly visible amounts in arterial blood. It was only when normal pigs were near death from severe venous gas embolism that visible arterial emboli were found (17). Undetectable gas emboli could of course pass undetected, as suggested by the present of gas phase in deep tissues in port mortem examinations.

Other pathophysiologic events occur prior to death. Peripheral vascular resistance increases and the blood plasma volume decreases (17). We have assumed these are effects of changes in capillary permeability and injury to peripheral vessels through undefined chemical or physical effects of peripheral gas lesions. No significant change in blood coagulation factors occurred, however, even when the animals were nearly dead. The eventual cause of death was respiratory failure with a shocklike syndrome. The gas bubble emboli do not appear to damage the pulmonary vascular endothelium even when steady state embolism is continuous for up to four hours (20). This differs from experiments using continuous venous infusion of air, where lung damage does occur (19).

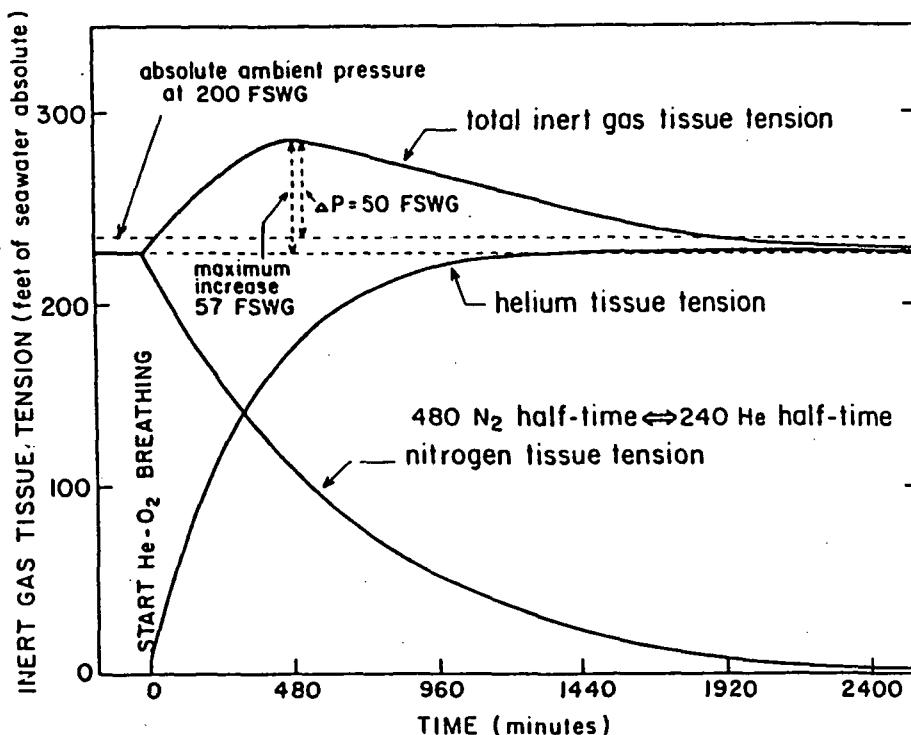


Fig. 7. Diagram of "deep tissue" isobaric supersaturation caused by breathing helium after prolonged exposure to N₂-O₂ mixture. Total inert gas pressure rises, and a composite supersaturation is maintained for many hours in "slow-perfusing" tissues. The opposite effect, an equivalent degree and time course of useful subsaturation, is expected with breathing N₂-O₂ after prolonged exposure to helium (4).

"Deep Tissue Isobaric Counterdiffusion"

Inert gas counterdiffusion in another form can create supersaturation in the deep tissues, even when the skin is underwater or not exposed to a different inert gas. "Deep Tissue Isobaric Counterdiffusion" was selected as the name to designate the supersaturation or subsaturation which occurs when a diver "switches" from breathing one inert gas-oxygen mixture to a different inert gas carrier for oxygen (4) (15). After such a switch, from a "slower equilibrating" to a "faster equilibrating" gas (e.g. from air to helium-oxygen) a slowly perfused tissue saturated with nitrogen will become transiently supersaturated by entry of the more rapidly exchanging helium. This deep-tissue supersaturation continues for many hours, as predicted (4) and as D'Aoust demonstrated by bubble detection in goats (15). The process has not been widely encountered in man, but has been observed in helium exposures following shallow nitrogen saturation (22). A long transient of venous gas emboli can develop (7) (15), and it should for practical purposes of diving safety be assumed until proven otherwise that gas lesions generated by decompression in poorly perfused tissues will be expanded by "deep tissue isobaric counterdiffusion" (2). It is for this reason that helium should not be substituted for air breathing in treatment of severe decompression sickness resulting from air diving, without the degree of concurrent compression calculated to assure marked immediate reduction of bubble volume (as in Treatment Table 7A (18).

The reverse principle can produce a beneficial "subsaturation," however, as Keller and Buehlmann proposed when they employed switch from faster to slower diffusing inert gases (e.g., helium to nitrogen) to accelerate overall inert gas elimination during decompression (16). Deep tissue isobaric inert gas counterdiffusion subsaturation is now used to advantage in several commercial and laboratory decompression procedures, as well as evolving military diving methods.

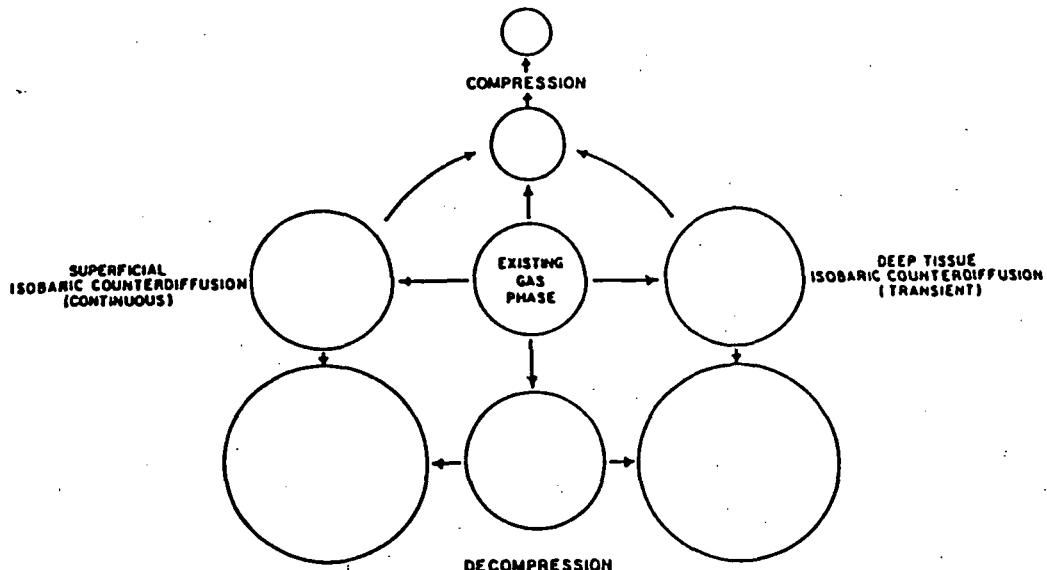


Fig. 8. Interactions among gas phase decompression, compression and isobaric states in several forms of gas lesion (2).

Interactions of Counterdiffusion Processes, Oxygen and Decompression

The concepts and empirical observations elaborated above illustrate that several types of gas lesion diseases are associated with diving, and can co-exist in two or more forms (1) (2). Decompression must necessarily exaggerate the effects of isobaric gas lesions, and isobaric supersaturations should also be conceived as necessarily exaggerating decompression incidents and the problems of decompression sickness therapy. In all, the informed use of increased oxygen pressures offers a means of prevention as well as a means of therapy (1). For the counterdiffusion diseases, absolute prevention of each is now possible through proper choice of respiratory and ambient gases or gas sequencing. Practical precautions recommended early to prevent or minimize adverse interactions include (2):

- ° Avoidance of mask breathing of air or N₂-O₂ while body surface or the ear canals are exposed to helium.
- ° Avoidance of an abrupt change from air or N₂-O₂ breathing to helium-oxygen at a constant or decreasing pressure.
- ° When it is clearly desirable during therapy for severe decompression sickness to proceed from air or N₂-O₂ breathing to prolonged exposure at a higher ambient pressure than is sensible for nitrogen, compression on helium is recommended despite the potential hazard of deep-tissue counterdiffusion supersaturation (18). Such change to helium should be accompanied by prompt compression and appropriate oxygen pressure, to counter the tendency for bubble growth or development.
- ° Switch from prolonged helium breathing to air or N₂-O₂ breathing to achieve deep counterdiffusion subsaturation is a desireable aid to prevention of decompression sickness in helium diving. In a pressure chamber it should occur by a change in ambient atmosphere rather than by mask breathing, to avoid superficial counterdiffusion. The transition should take place gradually, rather than abruptly, to avoid exaggerated sensations of narcosis which may be confused with vestibular effect.
- ° Use of increased oxygen in inert gas - oxygen mixtures remains the most effective means to reduce tissue total inert gas pressure in the prevention and therapy of each form of gas lesion disease. In conjunction with deep isobaric counterdiffusion sub-saturation the limitations are those of tolerance to oxygen.

REFERENCES

1. Lambertsen CJ. Relations of decompression to use of oxygen: harmful effects and their prevention. In: Symposium on health examination of Divers - decompression/use of oxygen. Norwegian Petroleum Directorate Report, Stavanger, 1988.
2. Lambertsen CJ (ed.) Decompression sickness and its therapy. Proceedings of a symposium organized by the Association of Diving Contractors and the Institute For Environmental Medicine, University of Pennsylvania. New Orleans, Louisiana, 1 February 1978. Allentown, PA: Air Products and Chemicals, Inc. 1979.
3. Lambertsen CJ, Gelfand R, Peterson RE, et al. Human tolerance to He, Ne and N₂ at respiratory gas densities equivalent to He-O₂ breathing at depths to 1200, 2000, 3000, 4000 and 5000 feet of sea water. Aviat Space Environ Med 1977; 48:843-855.
4. Lambertsen CJ, Idicula J. A new gas lesion syndrome in man induced by "isobaric counterdiffusion." J Appl Physiol 1975; 39:434-443.
5. Graves DJ, Idicula J, Lambertsen CJ, Quinn JA. Bubble formation resulting from counterdiffusion supersaturation: a possible explanation for isobaric inert gas 'urticaria' and vertigo. Phys Med Biol 1973; 18:356-364.
6. Blenkarn GD, Aquadro C, Hills BA, Saltzman HA. Urticaria following the sequential breathing of various inert gases at a constant ambient pressure of 7 ata: a possible manifestation of gas induced osmosis. Aerospace Med 1971; 42:141-146.
7. D'Aoust BG, Smith KH, Swanson HT, et al. Venous gas bubbles: production by transient deep isobaric counterdiffusion of helium against nitrogen. Science 1977; 197:889-891.
8. Cowley JRM, Allegra C, Lambertsen CJ. Subcutaneous tissue gas space pressure during superficial isobaric counterdiffusion. J Appl Physiol 1979; 47:224-227.
9. Idicula J, Graves DJ, Quinn JA, Lambertsen CJ. Bubble formation resulting from the steady counterdiffusion of two inert gases. In: Lambertsen CJ, ed. Underwater physiology V. Bethesda, MD: Federation of American Societies for Experimental Biology, 1976; 335-340.
10. Dueker CW, Lambertsen CJ, Rosowski JJ, Saunders JC. Middle ear gas exchange in isobaric counterdiffusion. J Appl Physiol 1979; 47:1239-1244.

11. Ranade A, Lambertsen CJ, Noordergraaf A. Inert gas exchange in the middle ear. *Acta Oto-Laryngol* 1980; 371 (Suppl.).
12. Mynderse LA, Lambertsen CJ. Superficial isobaric counterdiffusion of the round window in the guinea pig. Institute For Environmental Medicine, 1983.
13. Cowley JRM, Lambertsen CJ. Isobaric gas counterdiffusion in rabbit eye. *J Appl Physiol* 1979; 47:220-223.
14. Cunningham JPW, Lambertsen CJ. Quantitation of spontaneous intravascular gas embolism during superficial isobaric gas counterdiffusion. Institute For Environmental Medicine Report, 1981.
15. D'Aoust BG, Lambertsen CJ. Isobaric gas exchange and supersaturation by counterdiffusion. In: *The physiology and medicine of diving*, Third Edition, Bennett PB, Elliott DH, eds. London: Balliere-Tindall, 1983; 383-403.
16. Buhlmann AA. The use of multiple inert gas mixtures in decompression. In: *The physiology and medicine of diving and compressed air work*. Bennett PB, Elliott DH, eds. Baltimore, MD: Williams and Wilkins, 1969.
17. Pisarello JM, Fried M, Fisher D., Lambertsen CJ. Superficial isobaric counterdiffusion gas lesion disease - effects leading to mortality. In: *Underwater Physiology VIII*. Bachrach AJ, Matzen MM, eds. Bethesda: Undersea Medical Society, 1984; 101-106.
18. Lambertsen CJ, Miller RG. Treatment table 7A. Saturation decompression intermittent oxygen method for treatment of severe decompression sickness and gas embolism, or where onset of symptoms occurs prior to reaching surface. Institute For Environmental Medicine/Ecosystems/Solus Ocean Systems, 1980.
19. Albertine KH, Wiener-Kronish JP, Koike K., Staub ND. Quantification of damage by air emboli to lung microvessels in anesthetized sheep. *J Appl Physiol: Respirat Environ Exercise Physiol* 1984; 57(5):1360-1368.
20. Albertine KH, Lambertsen CJ. Effects of superficial isobaric counterdiffusion venous gas embolism upon lung in the pig. Institute For Environmental Medicine Report, 1988.
21. Harvey CA, Lambertsen CJ. Deep tissue isobaric inert gas exchange: predictions during normoxic helium, neon and nitrogen breathing by men at 1200 feet of sea water. In: *Underwater Physiology VI*. CW Shilling, Kent MB, eds. Bethesda: Federation of American Societies for Experimental Biology, 1979; 343-358.

Isobaric Counterdiffusion and Decompression

22. Harvey CA. Shallow saturation hyperbaric exposures to nitrogen-oxygen environments and isobaric switches to helium-oxygen. *Undersea Biomed Res* 1977; 4:A15.
23. Lambertsen CJ, Idicula J, Cunningham J, Cowley JRM. Pathophysiology of superficial isobaric counterdiffusion. *Institute For Environmental Medicine Report*, 1980.

ACKNOWLEDGEMENTS

The work performed at the Institute For Environmental Medicine was supported during various periods by the National Aeronautics and Space Administration (Grant NAS-9-17238-85), Naval Medical Research and Development Command (Contract N000 14-67-A-0216-0024) and by the National Heart, Lung and Blood Institute (HL 08899).

C. J. Lambertsen, M.D.
Institute For Environmental Medicine
1 John Morgan Building
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

APPENDIX B

Appendix B

Cardiovascular And Respiratory Responses
To Prolonged Superficial Isobaric Inert Gas
Counterdiffusion Gas Embolism In Pigs

J. B. Pisarello, C. J. Lambertsen and N.D. Flores

Project NAG 9-154 S5

Institute For Environmental Medicine
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

30 April 1991

IFEM DRAFT

CARDIOVASCULAR AND RESPIRATORY RESPONSES TO PROLONGED
ISOBARIC INERT GAS COUNTERDIFFUSION GAS EMBOLISM IN PIGS

Abstract

Anesthetized pigs were artificially ventilated with N₂O-O₂ while areas of body surface were exposed to helium at 1 ATA. Venous gas embolism generated by isobaric counterdiffusion (N₂O/He/1 ATA) was detected at a mean of 83 ± 23 min., by a doppler cuff placed around the inferior vena cava, and persisted until death or termination of the experiment. When only the hindlimbs of the animal were exposed to He (n=4), no change in baseline physiologic parameters occurred up to 18 hours despite the development of vena caval embolism. When the exposed skin area was increased to include trunk and extremities (n=9), pulmonary arterial hypertension occurred, without increase in pulmonary artery wedge pressures, but with progressive tachycardia, late systemic hypotension and decrease of cardiac output during the period of continued embolism. Sequential venous blood measurements demonstrated progressive hemoconcentration, no change in the normalized values of white cells, and a decrease in platelets. Sequential arterial blood gas determinations showed progressive hypoxemia, hypercapnia and acidosis. Survival ranged from 5.4 to 28.0 hours. At autopsy, all animals demonstrated large amounts of gas bubbles in the venous side of the circulation while 2 animals also demonstrated left ventricular and arterial free gas. In 5 additional animals, bronchoalveolar lavage material obtained at baseline and after 1 and 4 hours of embolism demonstrated no significant change in protein content or number or distribution of cells. In these animals, lung wet to dry weight ratios indicated no lung edema accumulation, and light microscopic

examination of post mortem lung tissue indicated preservation of the lung microstructure. Physiologic events observed during these lethal isobaric venous gas embolism exposures in pigs depended on the amount of skin exposed to the external gas. The isobaric phenomena resemble those in decompression sickness, with the important exception of the occurrence of pulmonary edema in severe pulmonary decompression sickness.

isobaric; inert gas; counterdiffusion; isobaric counterdiffusion; venous gas embolism; decompression sickness; gas embolism.

INTRODUCTION

Breathing an inert gas while surrounded by another of faster diffusive properties has been found in both man and animals to produce free gas in tissues, and venous gas emboli, without a change in environmental pressure (19). The process leading to the pathologic effects was named "Superficial Isobaric Counterdiffusion Gas Lesion Disease" by its discoverers (19). Its occurrence and severity has been shown to depend on the particular combination of respired and environmental gas as well as the environmental pressure (15). Its theoretical bases have been described (12)(16)(18)(19)(20)(21).

Initially identified in man, counterdiffusion supersaturation was found to produce severe itching, gas-filled dermal lesions, and vestibular derangement (19) (19a). In animals, prolongation of the process produces gas embolism and death (19a)(20)(28). Systematic analysis of different gas combinations and animal models has identified the young pig breathing N₂O while surrounded by helium as a useful experimental method for investigation of the effects of isobaric gas embolism at 1 ATA (15)(20). In this situation, gas bubbles develop in peripheral tissues and blood, ultimately resulting in death of the animal in few hours (19a)(20). After a latency period, the volume rate of isobaric venous gas embolism becomes nearly constant, is independent of the environmental pressure, and can be readily decreased by reduction of the inspired inert gas partial pressure (19a)(21). This experimental production of stable rates of isobaric gas embolism has provided a unique experimental model for systematic, dose-effect study of endogenously generated gas embolism in intact animals. It has special importance in

relation to the pathophysiology of decompression sicknesses and other dysbaric disorders, since the development of a gas phase in peripheral tissues and blood is a fundamental part of decompression pathophysiology (9)(19a). Isobaric counterdiffusion provides an alternative to production of bubbles by decompression, which is transient and unpredictable in amounts, or to intravenous infusion of gas bubbles, which ignores the contribution of gas lesion phenomena occurring in peripheral tissues. The purpose of the work presented in this paper was the analysis of circulatory and pulmonary effects during continuous N₂O/He isobaric counterdiffusion gas embolism leading to death.

METHODS

Animal Preparation

A total of 23 Yorkshire pigs of both sexes (20-30 kg) was utilized for the study. Animals were anesthetized with pentobarbital sodium (30-50 mg/kg i.p.) and placed in a prone position. Polyethylene catheters were placed into the left external jugular vein and common carotid artery, for administration of fluids to compensate for blood drawing and dermal and respiratory fluid losses, and for continuous recording of systemic arterial pressure. Adequate level of anesthesia was maintained with pentobarbital 5-10 mg/kg/hr intravenously, with additional intermittent administration to suppress spontaneous ventilation. A tracheal cannula was inserted through a cervical midline incision and the animal connected to a volume respirator. Ventilation gas was N₂O-O₂ 79/21%. Tidal volume and respiratory rate were adjusted to

keep PCO₂ within 36-40 torr. Ventilator settings were then held constant throughout the experiment. Sighing was accomplished during the experiment every 20 minutes with twice the tidal volume. An 8-French Swan Ganz thermodilution catheter was advanced through the right jugular vein to a branch of the pulmonary artery, guided by pressure contour. This allowed for continuous recording of pulmonary artery pressure and intermittent determination of thermodilution cardiac output (Edwards model 9510 A). Heart rate was derived from a continuously recorded lead II of the electrocardiogram. A Doppler bubble detector cuff (Parks Electronics Lab, Beaverton, Ore.) was then placed around the inferior vena cava through a lateral abdominal incision without entering the peritoneum. Doppler signals were analyzed in a model 1040 ultrasonic flowmeter (Sound Products: Institute for Applied Physiology and Medicine, Seattle, Washington), and displayed on an oscilloscope with audio output continuously monitored and recorded on magnetic tape. A thermal probe was utilized for continuous recording of rectal temperature, which was maintained between 1°C of initial baseline by intermittent use of a thermal blanket. The complete surgical procedure took approximately 30 minutes and blood loss was less than 25 ml.

Generation of Isobaric Counterdiffusion Gas Exchange

In all animals nitrous oxide with 21% oxygen was respired for 1 to 2 hours prior to exposing skin surface to helium. This control situation, used as a baseline, represented N₂O/Air/1 ATA counterdiffusion, a situation in which prior investigations have demonstrated no cutaneous gas lesions. In four early experiments, a plastic bag was placed around the hind limbs of the

animal and filled with helium, initiating the N₂O/He counterdiffusion process, as in a prior study (19a)(21). Since this limited body surface area for counterdiffusion did not produce definitive changes in measured physiologic parameters, the amount of exposed skin was increased to include the trunk and extremities in 9 additional animals. All data presented in this paper were obtained with this larger area of counterdiffusion, with only the head and neck exposed to air. In 5 control experiments, helium was not used and control measurements were performed repetitively for 12 hours.

Blood Sampling - Analytical Methods

During the baseline period and intermittently during the period of counterdiffusion, blood samples were collected via the jugular catheter for hematologic studies that included hemoglobin, hematocrit, white cell and platelet count by automated procedures with a cell track 560 (Angel Engineering, Nova, CT). Values were normalized based on changes in hemoglobin concentration. Blood gases were determined sequentially with a Corning 165/2 analyzer calibrated with radiometer precision buffers and laboratory validated gas mixtures. Measured blood values were corrected to rectal temperature. In 5 additional animals, bronchoalveolar lavage (BAL) material was obtained at baseline and after 1 and 4 hours of embolism, by infusion of 100 ml of normal saline in aliquots of 20 ml through a fiberoptic bronchoscope wedged in an appropriate position. Fluid aspirated following infusion was filtered through sterile gauze and was used for analysis of total protein and albumin content, as well as for total and differential cell counts.

After the death of the animal in the experimental counterdiffusion series and after 12 hours in the control experiments, the thoracic and abdominal cavities were opened and internal organs inspected for the presence and distribution of gas bubbles. Heart chambers and great vessels were opened. In 4 experimental animals, the left lung was taken out of the thorax and fixed overnight at 25 cm water pressure with buffered formalin. Sections were evaluated by light microscopy after hematoxylin and eosin staining. In 6 experimental animals and in 3 controls lung wet to dry weight ratios were obtained in the right lung.

Results

Cardiovascular, blood composition and blood gas values remained essentially at baseline levels in the control experiments evaluated for 12 hours using the N₂O/Air/1 ATA condition. Definite pathophysiologic effects occurred when N₂O/He/1 ATA counterdiffusion was produced. (Table 1, and Figures 1-8 in Final Report)

Dermal Changes and Survival. When only the hind limbs were exposed to helium, dermal lesions on the counterdiffused skin followed by vena caval embolism occurred as previously reported (21). However, no significant change from baseline levels of systemic or pulmonary arterial blood pressures occurred during exposure up to 18 hours. When the area of exposed skin was increased to involve trunk and extremities, the gas lesions of superficial counterdiffusion, beginning as flat erythematous blotches, appeared on the surface of the skin within 30 minutes of beginning exposure to helium, and

evolved in size and numbers to include the white, raised lesions previously described (20). Doppler signals of vena caval gas embolism developed at 1.5 ± 0.2 hours (mean \pm s.e.) (range 0.6 - 2.4) after the beginning of helium counterdiffusion exposure and persisted to the end of the experiment. In one animal, death occurred following the sudden development of systemic hypertension only 30 minutes after beginning helium flow into "counterdiffusion bag" surrounding the body. As the presence of a patent atrial septal defect was demonstrated at autopsy, this animal has not been included in the overall analysis which follows. Survival time of the other 8 animals was 10.3 ± 2.6 hours (range 5.4 - 28.0).

Circulatory Effects. No significant changes occurred prior to venous gas embolism. With the onset of intravascular bubbles, mean pulmonary artery pressure rose from a 17.3 ± 3.2 mm Hg control value to 28.4 ± 4.2 mm Hg and remained elevated until a circulatory failure associated with death. Pulmonary capillary wedge pressures remained at control levels or decreased slightly throughout the experiment. During the period of venous gas embolism, a progressive increase in heart rate was observed up to the time of death, associated with a decrease in cardiac output. Concurrently, a steady rise in hemoglobin concentration and hematocrit occurred, and a decrease in platelet count, while no significant changes occurred in the total white cell count (both normalized to initial hemoglobin concentration) (Fig. 4, 5). In 6 of the 8 animals, systemic arterial hypotension developed 1 to 2 hours prior to death. In 3 of these animals, infusion of 200 ml or normal saline over 10 minutes during the period of hypotension restored arterial blood pressure to normal levels. Discontinuation of the infusion resulted in return of

hypotension, and death. Two animals died rapidly without previous changes in systemic arterial pressure.

Arterial Blood Gases and pH. Blood gas and pH determinations during the period of counterdiffusion demonstrated progressive arterial hypoxemia, increase of PCO₂, and acidosis, in spite of constant volume artificial ventilation.

Broncho-Alveolar Lavage (BAL). Protein and albumin content, as well as differential and total blood cell counts in material collected during embolism, were not different from control values.

Necropsy. At necropsy, as in previous studies (19a)(20)(28), large amounts of free gas were noted in the venous side of most vascular beds, and in 2 animals gas bubbles were also seen in the left ventricle and coronary and systemic arteries. Measurements of lung wet to dry weight ratios of experimental animals were 6.1 ± 0.4 , not different than control values, and light microscopic examination of pulmonary tissue did not demonstrate abnormalities. Specifically, no perivascular cuffing or intra-alveolar material was found.

Discussion

Research following the discovery of the pathologic and lethal consequences of isobaric inert gas counterdiffusion gas lesion disease has demonstrated that as a result of this process, the characteristic continuous venous gas embolism may be accompanied by gas bubbles distributed extensively in tissues of multiple organs and systems (20). Our present experiments

determined the sequence of physiologic changes from the development of severe counterdiffusion venous gas embolism, to the death of the experimental animal. The results indicate that from initial gas embolism to death, multiple physiologic changes occur, and include derangements in blood composition, hemodynamics and gas exchange function.

A previous investigation, utilizing a venous gas embolism trap, has demonstrated that, once established, counterdiffusion venous gas embolism progresses at a stable rate (19a)(21). Thus, effects noted in the present experiments are considered to have been produced by a nearly constant "degree" of embolic stress. It was not possible in these experiments to both allow venous gas emboli to bombard the cardio/pulmonary/hematologic system and to trap the emboli for volume rate measurement. Although it is recognized that Doppler techniques do not provide a good quantitative measure of "embolic volume dose", the intensity of the signal indicating bubble frequency was noted to remain remarkably stable throughout the experiment. In addition, the stability of elevated pulmonary artery pressures throughout the embolic period suggest a constant embolic dose, since rate of venous gas embolism and degree of pulmonary hypertension have been elsewhere shown to be linearly correlated (7). When only hind limbs were exposed to helium, prolonged survival for up to 18 hours without significant variation in pulmonary artery and systemic artery pressures indicates that the lethal pathophysiological response to isobaric gas embolism increases with the amount of exposed gas. This capacity for controlling the rate of stable gas embolism in the superficial isobaric counterdiffusion model can be utilized in experiments requiring prolonged sublethal exposures such as in the study of late hematologic effects of

decompression from hyperbaric exposures (25).

Overall responses encountered during our experiments, including pulmonary arterial hypertension, systemic arterial hypotension, hemoconcentration, and arterial hypoxemia resemble previous findings in severe decompression-related gas lesion diseases in man and animals (8)(24).

Sustained pulmonary arterial hypertension has been previously reported repeatedly in association with gas embolism in circumstances like decompression sickness, experimental intravenous infusion of gas bubbles, and iatrogenic gas embolism (24)(13)(29). In addition to the obvious effect of obstruction of pulmonary vascular channels by bubbles, there is evidence from other models of venous gas embolism that humoral (17) and reflex (29) mechanisms may participate in the pathogenesis of the pulmonary arterial hypertension. Active vasoconstriction of pulmonary vascular beds not obstructed by bubbles has been suggested as a "protective mechanism" against development of pulmonary edema (13). The stability of pulmonary capillary pressures observed during counterdiffusion embolism in the present experiments essentially eliminates left heart failure as a contributory mechanism for the pulmonary hypertension. This is consistent with previous observations in other gas embolism syndromes (27) (2). Although the pulmonary hypertension induced by the effects of counterdiffusion venous gas embolism is comparable in degree to that produced by other experimental and clinical situations where definite lung injury occurs (15) (23) (1), our data concerning lung wet to dry weight measurements, analysis of BAL material, and lung light microscopic examination does not indicate that pulmonary edema was a prominent consequence of even lethal isobaric embolism. If present, lung injury produced by sub-

lethal isobaric counterdiffusion is mild, and any increases in lung microvascular permeability such as found in other models of gas embolism may be resolved by way of the pulmonary lymphatic drainage, limiting accumulation in the lung interstitium. Differences with effects on lung fluid balance noted previously with decompression or infusion of air bubbles (1)(8)(27) may relate to any of several factors including specific dimensional properties of bubbles, the background state and entity of inert gas saturation, and possibly reasons related to species differences in pulmonary response to gas embolism.

The observed impairment of pulmonary-arterial oxygen and carbon dioxide gas exchange function during the period of venous gas embolism is consistent with what has been observed previously by other investigators using different methods (8) (13). Such previous analysis in the dog has indicated that acute oxygen and carbon dioxide exchange impairment in gas infusion embolism is independent of the gas composition of the bubble; hypoxemia appears to be caused primarily by ventilation/perfusion mismatch rather than shunt (perhaps aggravated by decreases in cardiac output observed during our experiments), and increases in arterial PCO₂ relate to increments of physiologic dead space by addition of high ventilation/perfusion areas (13). In the present experiments with isobaric counterdiffusion, respiratory gas exchange alterations alone were insufficient to cause death. However, hypoxia may have been a contributory factor in damage of tissues with circulatory patterns altered by local gas lesions, and in the passage of venous gas emboli to the arterial side of the circulation (26).

A significant finding in these experiments relates to the presence of

widespread gas in the arterial blood of two animals in the series reported here. Although autopsy was performed immediately after the death of the animal, it is conceivable that some growth of the bubbles occurred even during that short period (19a). If this is the case, circulating bubbles before death may have been less in total volume. In any case, it is clear that at least some embolism must have occurred to the arterial side of the circulation and possibly for some time before death. The occasional transient arterial hypertension observed in these two animals during the isobaric embolic period may have been related to this phenomenon. The two animals that had arterial bubbles at autopsy had no grossly evident anatomical shunts. Neither did they demonstrate the profound cardiovascular changes commonly associated with arterial embolism (10), or differences in survival duration, or unusual cardiorespiratory phenomenon before death, compared to the rest of the animals. The passage of gas bubbles from the venous to the arterial circulation of dogs has been found to be dependent on the volume rate of embolism (5), and the size of the bubble (7). In our experiments, the transpulmonary passage of such emboli may have been caused by incomplete release of gas during alveolar transit, with small bubbles reaching the pulmonary veins and growing in the arterial circulation in the presence of saturated blood (19a). Another possibility relates to the opening of pulmonary vascular shunts during embolism. Such mechanisms of transpulmonary embolism have been documented and found to be aggravated by hypoxia (26), aminophylline (5) and oxygen toxicity (6). All are potential components of the pathology and treatment of the gas lesion diseases.

Determination of the conditions that produce and/or aggravate the

passage of undissolved gas from the venous to the arterial circulation is of critical importance in prevention of the dysbaric disorders. The counterdiffusion model provides a means of systematic investigation of this particular phenomenon.

The progressive hemoconcentration observed during our experiments has been a common finding in other situation where gas embolism exists. It has been interpreted as indicating a reduction in plasma volume as a result of increased microvascular permeability (2)(4). This effect has been observed in intact animals after rapid decompression (2), as well as in isolated venous and arterial strips (22). In the present study, the combined hemodynamic response of progressive tachycardia, decreased cardiac output and late hypotension is consistent with the development of progressive hypovolemia. In addition, during the period of hypotension it was possible to restore normal levels of systemic pressures with infusion of normal saline. Microvascular permeability increase was not found in the lung in these studies, at least as could be indicated by wet/dry weight ratios and light microscopy. This apparent paradox of venous gas embolism could relate to the fact that during counterdiffusion, persistent gas separation occurs in peripheral tissues (20) which could trigger and sustain the release of local inflammatory mediators with the capacity of amplification of this effect (19a). Bubbles could not be demonstrated in lung parenchyma during extensive studies of tissue distribution of counterdiffusion bubbles (20). The observed decrease in platelet counts during the late phase of isobaric embolism, previously observed in association to decompression-related phenomena (25), indicates that these cells participate in the effects of counterdiffusion gas embolic

processes.

In conclusion, the present experiments demonstrated the occurrence of changes in blood composition, circulation and respiration during continuous venous gas embolism generated by lethal isobaric counterdiffusion. Abnormalities in pulmonary gas exchange appear due to functional rather than structural disruption. In addition, it was found that during severe counterdiffusion, when critical conditions develop, bubbles can reach the left side of the circulation even without an abnormal anatomical shunt, and may possibly be tolerated for a period of time, then constitute one of the potential mechanisms of death in counterdiffusion embolism. The hemodynamic response of progressive tachycardia, decrease in cardiac output and late arterial hypotension associated with progressive hemoconcentration strongly suggest the development of peripheral systemic microvascular plasma loss resulting in lethal hypovolemia. Isobaric counterdiffusion has provided a method for systematic investigation of biological effects of endogenously generated gas embolism in gas lesion diseases. Characterization of the basic mechanisms operative in this complex group of diseases will provide a rational basis for improving upon present techniques of prevention and therapy.

TABLE 1

(Appendix B)

HEMODYNAMIC, BLOOD GAS AND HEMATOLOGIC RESPONSE
TO SUPERFICIAL ISOBARIC COUNTERDIFFUSION VENOUS GAS EMBOLISM

<u>Observation</u>	<u>Animals</u>	<u>Baseline</u>	<u>Counterdiffusion</u>	
			<u>2.4 Hours</u>	<u>5.2 Hours</u>
HR	Beats/m	6	148.3 ± 5	186 ± 10*
PAP	mm Hg	6	17.4 ± 1.4	33.1 ± 4.5*
PVR	cm H ₂ O/L/m	6	4.5	10.8
SP	mm Hg	6	105.8 ± 2.0	105.1 ± 2.3
CO	L/m	5	4.52 ± 0.73	3.82 ± 0.74
PO ₂	mm Hg	5	87.5 ± 0.5	56.1 ± 4.0*
PCO ₂	mm Hg	5	38.1 ± 1.2	44.3 ± 2.8
pH		5	7.48 ± 0.02	7.39 ± 0.04
HGB	gm/dl	5	11.1 ± 0.7	12.1 ± 0.4
HCT	%	5	34.1 ± 1.1	36.6 ± 2.1
WBC	× 10 ³ /ml	5	19.6 ± 2.9	23.2 ± 5.2
NEUT.	%WCB	5	43.6 ± 7.4	59.0 ± 10.2
PLAT	× 10 ³ /ml	5	576 ± 94	508 ± 67
				436 ± 97*

HR - Heart Rate; PAP - Pulmonary Artery Pressure; PVR - Pulmonary Vascular Resistance; SP - Systolic Pressure; CO - Cardiac Output; PO₂ - arterial partial pressure of oxygen; PCO₂ - arterial partial pressure of CO₂; HGB - Hemoglobin; HCT - Hematocrit; WBC - White Blood Cell Count; Neutrophils; Plat - Platelet Count.

* = significant change from Baseline (p < .05)

References

1. Albertine, K.H., J.P. Wiener-Kronish, K. Koike and N.C. Staub. Quantification of damage by air emboli to lung microvessels in anesthetized sheep. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57(5):1360-1368, 1984.
2. Bove, A.A., J.M. Hallenbeck and D.H. Elliott. Circulatory responses to venous embolism and decompression sickness in dogs. Undersea Biomed. Res. 1, 207-220, 1974.
3. Bove, A.A. The basis for drug therapy in decompression sickness. Undersea Biomedical Research Vol. 9, No. 2, June 1982.
4. Brunner, F.P., P.G. Frick and A.A. Buhlmann. Post decompression shock due to extravasation of plasma. Lancet 1, 1071-1073, 1964.
5. Butler, B.D. and B.A. Hills. The lung as a filter for microbubbles. J. Appl. Physiol. 47(3): 537-543, 1979.
6. Butler, B.D. and B.A. Hills. Effect of excessive oxygen upon the capability of the lungs to filter gas emboli. In: Underwater Physiology VII, edited by A.J. Bachrach and M.M. Matzen. Bethesda, MD: Undersea Med. Soc., 95-102, 1981.
7. Butler, B.D. and B.A. Hills. Transpulmonary passage of venous air emboli. J. Appl. Physiol. 59(2):543-547, 1985.

8. Catron, P.W., E. Flynn, Jr., L. Yaffe, M. Bradley, L. Thomas, D. Hinman, S. Survanshi, J. Johnson and J. Harrington. Morphological and physiological responses of the lungs of dogs to acute decompression. J. Appl. Physiol. 57(2):467-474, 1984.
9. Elliott, D.H. and E. Kindwall. Manifestations of decompression disorders. In: The Physiology and Medicine of Diving, 3rd ed. edited by P.B. Bennett and D.H. Elliott. London: Bailliere Tindall, 461-472, 1982.
10. Evans, D.E., A.I. Kobrine, P.K. Weathersby and M.E. Bradley. Cardiovascular effects of cerebral air embolism. Stroke 12, 338-344, 1981.
11. Ferris, E.B. and G.L. Engel. The clinical nature of high altitude decompression sickness. In: Decompression Sickness, Caisson Sickness, Diver's and Flier's Bends and Related Syndromes, edited by J.F. Fulton. Philadelphia: W.B. Saunders Co, 4-52, 1951.
12. Graves, D.J., J. Idicula, C.J. Lambertsen and J.A. Quinn. Bubble formation in physical and biological systems: A manifestation of counterdiffusion in composite media. Science 179: 582-584, 1973.

13. Hlastala, M.P., H.T. Robertson and B.K. Ross. Gas exchange abnormalities produced by venous gas emboli. Respir. Physiol. 36(1): 1-17, 1979.
14. Hjelle, J.O. Arterial Bubbles during decompression in a deep saturation dive. Undersea Biomedical Research, (suppl.) vol. 14, No. 2, March 1987.
15. Idicula, J., D.J. Graves, J.A. Quinn and C.J. Lambertsen. Bubble formation resulting from the steady counterdiffusion of two inert gases. In: Underwater Physiology V. Lambertsen, C.J. (ed.). Bethesda, MD: FASEB, 1976. pp. 335-340.
16. Imbert, J.P. Proposition of a perfusion limited model for isobaric counterdiffusion. M.S. thesis, University of Pennsylvania, 1975.
17. Johannes, M, A. Tucker, E.K. Weir, J.T. Reeves and R.F. Grover. Platelet-mediated pulmonary hypertension and hypoxia during pulmonary microembolism. Chest, 74:6, 1978.
18. Karreman, G. and C.J. Lambertsen. Kinetics of isobaric counterdiffusion. Bull. Mathematical Biol. 39: 587-595 1977.

19. Lambertsen, C.J., and J. Idicula. A new gas lesion syndrome in man induced by "isobaric gas counterdiffusion." J. appl. Physiol. 39: 434-443, 1975.
- 19a. Lambertsen, C.J. Relations of isobaric gas counterdiffusion and decompression gas lesion diseases. In: Vann, R.D., Ed. The Physiological Basis of Decompression: UHMS Publ. 75 (Phys) Bethesda MD: Undersea and Hyperbaric Med. Soc., 87-106, June 1989.
20. Lambertsen, C. J., and J. Idicula. Cutaneous gas lesions and continuous, lethal gas embolization in animals due to isobaric inert gas counterdiffusion. Fed. Proc. 33: 455, 1974.
21. Lambertsen, C.J., J.P.W. Cunningham and J.R.M. Cowley. The dynamics and composition of spontaneous, continuous gas embolism in the pig during isobaric gas counterdiffusion Fed. Proc. 34: 452, 1975.
22. Levin, L.L., G.J. Stewart, P.R. Lynch and A.A. Bove. Blood and blood vessel wall changes induced by decompression sickness in dogs. J. Appl. Physiol.; Respirat. Environ. Exercise Physiol. 50:944-949, 1981.
23. Moosavi, H., H.J. Utell, R.W. Hyde, P.J. Fahey, B.T. Peterson, J. Donnelly and K.D. Sensen. Lung ultrastructure in noncardiogenic pulmonary edema induced by air embolization in dogs. Lab. Invest. 45(5): 456-464, 1981.

24. Neuman, T.S., R.G. Spragg, P.D. Wagner and K.M. Moser. Cardiopulmonary consequences of decompression stress. Respir. Physiol. 41:143-153, 1980.
25. Neumann, T.S., R.G. Spragg and H. Wohl. Platelet aggregates following decompression. Undersea Biomed. Res. 8(Suppl.), 42, 1981.
26. Niden, A.H. and D.M. Aviado, Jr. Effects of Pulmonary embolism on the pulmonary circulation with special reference to arteriovenous shunts in the lung. Circ. Res. 4:67-73, 1956.
27. Ohkuda, K., K. Nakahara, A. Binder, and C. Staub. Venous air emboli in sheep: reversible increase in lung microvascular permeability. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51, 887-894, 1981.
28. Pisarello, J., M. Fried, D. Fisher and C.J. Lambertsen. Superficial isobaric counterdiffusion gas lesion disease - effects leading to mortality. In: Proceedings of the Eighth Symposium on Underwater Physiology. Bethesda: Undersea Medical Society, 101-106, 1984.
29. Yoshida, M., I. Sekalay, H.H. Rotman and P. Kimbel. Pulmonary vascular changes following air embolism in the dog. Archives Internationales de Physiologie et de Biochime, 86, 317-325, 1978.

APPENDIX C

APPENDIX C

Effects of Non-Lethal Degrees of
Isobaric Counterdiffusion Venous Gas
Embolism on Blood Contact System
and Coagulation.

J.B. Pisarello, N.D. Flores,
R.W. Coleman and C.J. Lambertsen.

Project NAG 9-154 S5

Institute For Environmental Medicine
University of Pennsylvania Medical Center
Philadelphia, PA 19104-6068

1 January 1990

IFEM DRAFT

Abstract

Experiments were performed to study the effect of isobaric counterdiffusion venous gas embolism on the blood contact system and coagulation. Counterdiffusion venous gas embolism was generated by helium exposure of trunk and extremities of 6 anesthetized instrumented pigs artificially ventilated with N₂O/O₂ at 1 ATA. Extensive dermal changes, pulmonary artery hypertension, tachycardia, decrease in cardiac output, arterial hypoxemia, and hemoconcentration occurred during the embolic period in all animals. Values of PT, PTT, Plasma Fibrinogen concentration, and functional activities of factors XII, XI, Pk (prekallikrein) and HMWK (High Molecular Weight Kininogen) did not significantly change from baseline values after 1 and 4 hours of continuous embolism. In 2 of these animals, samples collected after 6 hours of embolism during systemic hypotension preceding death, also demonstrated no changes in these parameters. We concluded that lethal pathophysiologic phenomena occurring during isobaric counterdiffusion gas embolism are not dependent upon activation of the blood contact system and coagulation.

Gas embolism; decompression sickness; contact system; coagulation; bubble-blood interaction; isobaric counterdiffusion; diving; aerospace.

Introduction

The presence of gas bubbles in tissues and blood of man and animals is known to produce pathological manifestations in a variety of clinical, operational and experimental situations (1)(18)(34). In addition, gas "bubble" surfaces are thought to play a central role in the generation of decompression sickness, a pathological state of varied symptomatology associated with reduction of environmental pressure during undersea and aerospace operations (6)(7)(19).

In addition to causing obstruction of vascular channels, it is considered that part of the pathogenesis of gas lesion diseases relates to the biological responses elicited by gas bubbles in peripheral tissues and blood (10)(5). Platelet and neutrophil aggregation (2), endothelial damage (32), complement activation (40) and occurrence of a beneficial therapeutic response to anti-inflammatory agents (8) are some of the observations that support this notion. Knowledge of the pathways of activation of such responses is of importance for the development of methods of prevention and therapy of gas lesion diseases. However, the initiating events and the specific biological defense systems participating in the development of these responses have not as yet been identified. Possible initiating factors include release of mediators by mechanical injury of tissues, stimulation of cellular components of blood, and activation of humoral circulating systems by bubbles themselves or bubble mediated tissue structural damage.

One of the systems that has been postulated as an active participant in the initial phase of the pathogenesis of dysbaric disorders is the blood contact

system (13). This group of proteolytic enzymes initiates surface activation of coagulation, fibrinolysis complement and kinin formation and has been found to participate in many human inflammatory and immunologic diseases including arthritis (23), transfusion reactions (3), renal allograft rejection (14) and thyroid and rocky mountain spotted fevers (15)(42) and nephritic syndrome (29). The participation of the contact system in the pathogenesis of gas lesion diseases has been suggested by in vitro (24) and in vivo (25) experiments as well as observations in man and animals consistent with consumption coagulopathy in severe cases of decompression sickness (35)(5). However, and in vivo detailed analysis of this system during the initial period of endogenously generated venous gas embolism is not available. The pig isobaric counterdiffusion gas exchange model (36) provides an excellent opportunity to perform this type of experiment, since it provides a stable amount of tissue-generated gas embolism associated with physiologic changes typical of gas lesion disease (26)(37). Of additional importance is that the pig contact system resembles the human (41).

This paper presents the results of experiments designed to study the participation of the contact and coagulation systems in the pathophysiologic responses to prolonged, continuous isobaric counterdiffusion venous gas embolism in pigs.

Methods

Animal preparation. This preparation has been described previously in detail (26)(36). Briefly, Yorkshire pigs (25-35 kg) anesthetized with pentobarbital were instrumented with vascular catheters for continuous recording

of common carotid artery pressures and a left external jugular vein line for administration of fluids and blood sampling. Artificial ventilation was accomplished with N_2O/O_2 (79%/21%) with a tidal volume of 15 ml/kg. The respiratory rate was initially adjusted to produce a PCO_2 of 36-40 torr and this setting was held constant throughout the experiment. An 8 French Swan Ganz catheter was advanced through the right jugular vein guided by pressure contour into the pulmonary artery or one of its branches for continuous recording of pulmonary artery pressures and intermittent determination of cardiac output (Edwards model 9510A). To initiate the counterdiffusion process, a plastic bag was placed around the trunk and extremities and filled with flowing helium. In 3 control experiments, helium was omitted and measurement followed for 8 hours.

Experimental protocol. The time of development of venous gas embolism (about 80 minutes from beginning helium flow) was considered the initiation of pulmonary hypertension, as a close correlation of these parameters has been previously observed a close correlation of these parameters (36). At baseline and after 1 and 4 hours of embolism, blood samples were collected from the jugular vein catheter for hematologic studies that included hemoglobin, hematocrit, white cell and differential and platelet count by automated procedures with a cell track 560 (Angel; Engineering, Nova, CT). Values of white cell and platelet concentrations were normalized to baseline hemoglobin. Simultaneously, arterial blood gases were determined with a Corning 165/2 analyzer calibrated with radiometer precision buffers and analyzed gas mixtures. Measured values were corrected to rectal temperature.

Functional activity of contact factors (activated partial thromboplastin time, (aPTT), prothrombin time (PT) and fibrinogen concentration) was determined

in venous samples in all animals and values normalized by the method of Dill (17). In addition, for the purpose of comparison, in 2 animals simultaneous arterial samples were obtained for measurement of blood cell counts and contact factor activity.

In 2 of the animals in this overall group it was possible to collect additional samples after 6 hours of continuous gas embolism, at which time preterminal systemic hypotension was present.

Hemodynamic parameters were followed in all animals until death occurred. Immediately following (within two minutes), and autopsy was begun that included inspection of all major organs, and presence of bubbles in vascular beds.

Analytical methods. For determination of activity of contact factors and coagulation times, 4.5 ml of blood were collected in 0.5 ml of 3.8% sodium citrate-0.1M e-aminocaproic acid. Samples were spun at 10,000 g for 15 min. in a refrigerated centrifuge, divided in 0.3 ml aliquots and stored at -70* until processing. Only polypropylene materials were utilized for collection and handling of samples. Samples for Pk (Prekallikrein) determination were processed at room temperature before being stored in a deep freezer, as there is indication that cold is capable of activating this protein. The aPTT, PT and fibrinogen concentration were determined according to standard methods (12)(8)(38). Functional assays of factors XII, XI, and High Molecular Weight Kininogen (HMWK) were measured with a modified aPTT utilizing factor deficient human plasma. PK activity was measured with a chromogenic assay (16).

Results

Control experiments showed no alteration in hemodynamics, hematologic or blood gas values followed during 8 hours. In the experimental animals, as previously described, (26) erythematous lesions appeared on the surface of the skin within 30 minutes of the beginning of counterdiffusion, followed by gas filled lesions mainly on the skin of abdomen, thorax and outer aspects of the limbs. After an average of 85 minutes, mean pulmonary artery pressures increased from a baseline of $17.4 +/ - 2.0$ mm.Hg. to $33.0 +/ - 4.55$ (mean $+/-$ s.e.) signaling the initiation of venous gas embolism (36). Pulmonary artery hypertension persisted at an elevated level until death of the animal, associated with progressive tachycardia and a decrease in cardiac output, while systemic arterial pressures remained close to control levels almost to death during the period of embolism in 4 animals but decreased by the 6th hour and until death in 2 animals. Details of cardiovascular and respiratory changes, and findings at necropsy are described in a parallel paper (37).

Hemoglobin concentration and hematocrit increased significantly at 1 and 4 hours. Based on these data, reductions in plasma volume of 15% and 26% respectively were calculated by the method of Dill (17), and utilized to normalize information concerning coagulation factors. White cell counts did not change significantly from baseline levels for all animals at 1 and 4 hours, or in the 2 animals studied for 6 hours. Platelet counts decreased with prolonged exposure.

Significant alterations did not occur in the normalized values of aPTT,

PT, fibrinogen levels or functional activity of factors XII, XI, PK and Hmwk obtained at 1 and 4 hours of counterdiffusion embolism.

Discussion

Many of the clinical manifestations of gas lesion diseases in man and animals suggest the participation of inflammatory processes in the development of pathophysiologic effects (5)(10). In addition, intact animal experiments have demonstrated the capacity of gas bubbles to effect tissue injury through mechanisms ancillary to direct physical trauma or vascular blockage. Selective depletion of cell populations (20) and inhibition of specific mediators (21)(22)(28) have been shown to modulate local pathophysiologic effects of gas bubbles, and thus suggested their participation in the pathologic process. However, the nature, precise sequence of generation and relative importance of inflammatory species in dysbaric disorders are not known.

The participation of the contact system in the initial steps of the pathogenesis of gas embolic disorders, resulting in the development of a harmful response, has been postulated based on in vitro and in vivo observations (24)(31)(27). Denaturation of plasma proteins on the surface of gas bubbles demonstrated by Lee (30), provides the potential substrate for activation of factor XII, the first step in the chain of events leading to activation of the coagulation, complement and kinin formation systems. In addition, an intermediate product of this activation factor XIIa, has been shown to have the ability to activate neutrophils (39), which may explain the aggregation of platelets and neutrophils observed frequently on the bubble surface (2) (32).

In addition, plasma of dogs and selective populations of rabbits in contact with gas bubbles demonstrate activation of the coagulation (24) and complement (40) systems respectively, processes potentially mediated by the contact system. Further support for the notion of surface activation of biological defense systems by bubbles is provided by documentation of coagulation abnormalities in severe cases of decompression sickness in divers (5) and experimental animals (35).

However, our experiments failed to detect a significant activation of these systems which is usually manifested by a decrease in coagulation 34 mm gas, after a prolonged period of tissue-generated venous gas embolism associated with dermal and subcutaneous tissue disruption, hemodynamic changes, hypoxemia and hemoconcentration. It is therefore unlikely that in our experimental situation, isobaric counterdiffusion venous gas embolism, the blood contact system is responsible for the initiation of the processes leading to these gross pathophysiologic effects which actually occurred in prolonged exposures. Direct stimulation of leukocytes and platelets and activation of humoral systems independent of the contact system remain as possible alternative mechanisms.

The present experiments were purposely limited to the embolic early (one to four hour) period associated with hemodynamic stability, and avoided the near death period with its profound changes in multiple physiological parameters. These gross effects would have made interpretation of positive changes in the contact system difficult. However, analysis of blood samples collected in 2 animals during the hypotensive period that preceded death also demonstrated no activation of contact factors even at this advanced stage of the lethal gas

embolism. This supports the concept that hypotension was generated in this situation through mechanisms independent of increased generation of bradykinin in the superficial sites of gas lesion development.

The present results are comparable to what was reported by O'Bradovich (33) in experiments where increased pulmonary microvascular permeability occurred during air embolism in sheep and in dogs on the basis of endothelial damage (2) independently of the activation of the contact system. Previous observations in sheep of pulmonary damage produced by intravenous infusion of air bubbles have similarly suggested the absence of activation of the coagulation system in this particular experimental situation (9).

The degree of contribution of surface activation of blood and cells in the pathogenesis of dysbaric disorders in man remains unsettled. While our results indicate the lack of activity of the system in our particular experimental situation, differences exist between counterdiffusion venous gas embolism and other forms of gas embolism. These include bubble size and composition, sites of origin and rate of generation, and the still unexplained absence of visible pulmonary injury in this model. It is not conceivable that initial aspects of gas lesion formation in the pathogenesis of decompression sickness and isobaric embolism are fundamentally different, even though gases may differ. The lack of participation of the contact system in gas bubble-mediated injury has also been documented in other animals (sheep and dog), in situations in which the composition of the infused bubbles is identical. This supports the concept that surface generated defense responses do not constitute a primary and general pathogenic phenomenon of mild degrees of

dysbaric disorders. The observations of coagulation abnormalities in severe cases of human decompression sickness could then be interpreted as secondary, rather than as an initiating phenomenon.

The mechanisms leading to the multiple pathogenic effects observed in the gas lesion diseases are complex, and elucidation of the contribution of these mechanisms remains of critical importance in the rational prevention and treatment of these related diseases. Isobaric counterdiffusion gas lesion generation has provided an excellent experimental tool for the in-vivo investigation of these mechanisms. Under the experimental conditions, physiologic disruptions associated with extensive distribution of gas bubbles in tissue and blood have been found to occur independent of activation of the contact system and coagulation.

TABLE 1

(Appendix C)

BLOOD CONTACT AND COAGULATION FACTORS
IN SUPERFICIAL ISOBARIC VENOUS EMBOLISM

<u>Observation</u>	<u>Animals</u>	<u>Baseline</u>	<u>Counterdiffusion</u>	
			<u>2.4 Hours</u>	<u>5.2 Hours</u>
Factor XIII (U/ml)	6	0.54 ± 0.06	0.76 ± 0.12	0.51 ± 0.06
Factor XI (U/ml)	4	1.21 ± 0.04	1.14 ± 0.08	0.90 ± 0.12*
Prekallikrein (U/ml)	5	0.91 ± 0.02	0.83 ± .12	0.65 ± 0.10*
High Mol Wt Kininogen (U/ml)	6	1.48 ± 0.12	1.54 ± 0.19	1.17 ± 0.22
Partial Thromboplastin Time (sec)	-	23.8 ± 1.6	23.2 ± 2.3	24.4 ± 4.0
Prothrombin Time (sec)	6	9.9 ± 0.3	9.9 ± 0.2	10.1 ± 0.4
Fibrinogen (mg/dl)	-	156 ± 24	170 ± 29	214 ± 28*

* = significant change from Baseline ($p < .05$)

REFERENCES

1. Abernathy, C.M. et al. Massive air emboli from intravenous infusion pump: etiology and prevention. Am. J. Surg. 137(2): 274-5, 1979.
2. Albertine, K.H., J.P. Wiener-Kronish, K. Koike and N.C. Staub. Quantification of damage by air emboli to lung microvessels in anesthetized sheep. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57(5):1360-1368, 1984.
3. Alving B., J. Hojima, J. Pisano., B. Mason, R. Buckingham, Jr., M. Mozen, and J. Finlayson. Hypotension associated with prekallikrein activator (Hageman-factor fragments) in plasma protein fraction. N. Engl. J. Med., 299, 66, 1978.
4. Anderson, J.C. and P.B. Bennett. Hematologic change. Acute decompression sickness. Lancet 2, 1193-1199, Biomed. Res. 8 (Suppl.), 43, 1981.
5. Anderson, J., and J. Hallenbeck. Pathogenesis of the Decompression Disorders. In: The Physiology and Medicine of Diving. Edited by Bennett and Elliot, Best Publishing Co., San Pedro, CA, 1982.
6. Behnke, A.R. Decompression sickness following exposure to high pressures. In: Decompression Sickness. Caisson Sickness, Diver's and Flier's Bends and Related Syndromes, edited by J.F. Fulton: Philadelphia: W.B. Saunders Co., 1951, p. 53-89.
7. Berghage, T.E. Decompression sickness during saturation dives. Undersea Biomed. Res. 3, 387-398, 1976.
8. Biggs, R.G., R.G. Macfarlane. Human blood coagulation. Haemostasis and Thrombosis, 2nd edn. Blackwell Scientific Publications, Oxford, 1976.
9. Binder, A.S., K. Nakahara, K. Ohkuda, W. Kageler, and N.C. Staub. Effect of heparin or fibrinogen depletion on lung fluid balance in sheep after emboli. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 47:213-219, 1979.
10. Bove, A.A. The basis for drug therapy in decompression sickness. Undersea Biomedical Research Vo. 9, No. 2, June 1982.
11. Catron, P.W., E. Flynn, Jr., L. Yaffe, M. Bradley, L. Thomas, D. Hinman, S. Survanshi, J. Johnson and J. Harrington. Morphological and physiological responses of the lungs of dogs to acute decompression. J. Appl. Physiol. 57(2):467-474, 1984.
12. Clauss, A. Gennnunssphysiologische schenell methode zuv bestimmons dostibninogens. Acta Hematol., 17: 237-246, 1957.

13. Colman, R.W. and A.M. Schmaier. The contact activation system: Biochemistry and interactions of those surface-mediated defense reactions. In: Cavis 5th ed. CRC Critical Reviews in Oncology/Hematology. Boca Raton, FL CRC Press Inc, 57-85, 1986.
14. Colman, R.W., G. Girey, E.G. Galvanek, and G.J. Busch. Human renal allografts: the protective effects of heparin, kallikrein activation and fibrinolysis during hyperacute rejection, in Symposium on Coagulation Problems in Transplanted Organs, Kaulla, K.V., Eds., Charles C. Thomas, Springfield, Ill., 87, 1972.
15. Colman, R.W., R. Edelman, C.F. Scott, R.H. Gilman, et al. Plasma kallikrein activation and inhibition during typhoid fever. J. Clin. Invest., 61, 287, 1978.
16. DeLa Cadena, R.A., C.F. Scott, and R.W. Colman. Evaluation of a microassay for human plasma prekallikrein. J. Lab. Clin. Med., 109: 601-607, 1987.
17. Dill, D.B., and D.I. Costill. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J. Appl. Physiol. 37(2): 247-248, 1974.
18. Ence, T.J. et al. Adult respiratory distress syndrome after venous air embolism. Am. Rev. Respir. Dis. 119(6): 1033-1037, 1979.
19. Ferris, E.B. and G.L. Engel. The clinical nature of high altitude decompression sickness. In: Decompression Sickness. Caisson Sickness, Diver's and Flier's Bends and Related Syndromes, edited by J.F. Fulton. Philadelphia: W.B. Saunders Co, 4-52, 1951.
20. Flick, M.R., A. Perel and N.C. Staub. Leukocytes are required for increased lung microvascular permeability after microembolization in sheep. Circ. Res. 48: 344-351, 1981.
21. Flick, M.R., J.M. Hoeffel, and N.C. Staub. Superoxide dismutase with heparin prevents increased lung vascular permeability during air emboli in sheep. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55(4):1284-1291, 1983.
22. Fukushima, M., and T. Kobayashi. Effects of thromboxane synthase inhibition on air emboli lung injury in sheep. J. Appl. Physiol. 60(6):1828:33, 1986.
23. Ginsberg, M.H., B. Jaques, C.G. Cochrane, and J.H. Griffin. Crystal-dependent cleavage of Hageman factor in human plasma and synovial fluid. J. Lab. Clin. Med., 95, 497, 1980.
24. Hallenbeck, J. M., A.A. Bove, R.B. Moquin and D.H. Elliott. Accelerated coagulation of whole blood and cell-free plasma by bubbling in vitro. Aerospace. Med. 44: 712-714, 1973.

25. Hallenbeck, J.M., A.A. Bove, and D.H. Elliott. Mechanisms underlying spinal cord damage in decompression sickness. Neurology 25: 308-316, 1975b.
26. Idicula, J., D.J. Graves, J.A. Quinn and C.J. Lambertsen. Bubble formation resulting from the steady counterdiffusion of two inert gases. In: Underwater Physiology V. Lambertsen, C.J. (ed.). Bethesda, MD: FASEB, 1976. pp. 335-340.
27. Inwood, M.J. and R.B. Philip. Experimental evidence in support of the hypothesis that intravascular bubbles activate the hemostatic mechanism. In: Blood-Bubble Interactions in Decompression Sickness, pp. 171-197, CDIEM Conf. Report 73-CP-960, Toronto 1973.
28. Ishihara, Y., Y. Uchida, and S. Kitamura. Effect of thromboxane synthetase inhibitors (OKY-046, OKY-1580) on experimentally induced air embolism in anesthetized dogs. Prostaglandins Leukotrienes Med. 21(2):197-206, 1986.
29. Kallen, R.J., and L. Soo-Kwang. A study of the plasma kinin generating system in children with the minimal lesion idiopathic nephrotic syndrome. Pediatr. Res. 9, 705, 1975.
30. Lee, W.H., Jr. and P. Hairston. Structural effects on blood proteins at the gas-blood interface. Fedn. Proc. 30: 1615-1622, 1971.
31. Lethosalo, J., T. Tervo, and L.A. Laitinen. Gas bubbles and platelet aggregation in the cerebral veins during acute decompression sickness. Undersea Biomed. Res. 8 (Suppl.), 42.
32. Moosavi, H., M.J. Utell, R.W. Hyde, P.J. Fahey, B.T. Peterson, J. Donnelly, and K. DeMesy Jensen. Lung ultrastructure in noncardiogenic pulmonary edema induced by air embolization in dogs. Lab. Invest. 45(5): 456-464, 1981.
33. O'Brodovich, H., M. Andrew, R. Silver, and G. Coates. Assessment of coagulation cascade during air microembolization of the lung. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55(6):1743-1747, 1983.
34. Ohkuda, K., K. Nakahara, A. Binder, and C. Staub. Venous air emboli in sheep: reversible increase in lung microvascular permeability. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51(4), 887-894, 1981.
35. Philip, R.B., P. Schalham, and C.W. Gowdey. Involvement of platelet and microthrombi in experimental decompression sickness: similarities with disseminated intravascular coagulation. Aerospace Med. 42:494, 1971.

36. Pisarello, J., M. Fried, D. Fisher and C.J. Lambertsen. Superficial isobaric counterdiffusion gas lesion disease - effects leading to mortality. In: Proceedings of the Eighth Symposium on Underwater Physiology. Bethesda: Undersea Medical Society, 101-106, 1984.
37. Pisarello, J., C.J. Lambertsen and N.D. Flores. Cardiovascular and respiratory responses to prolonged isobaric counterdiffusion embolism in pigs. Institute For Environmental Medicine Report, 20 March 1991.
38. Quick, A.J. "The one-stage prothrombin time". In: Human Blood Coagulation: Haemostosis and Thrombosis, 2nd edn. Blackwell Scientific Publications, Oxford, p. 609, 1976.
39. Wachtfogel, Y.T., R.A. Pixley, U. Kuchich, W. Abrams, G. Weinbaum, M. Schapira, and R.W. Colman. Purified human plasma factor XIIa aggregates human neutrophil and causes degranulation. Blood 67:1731-1737, 1986.
40. Ward, G.A., A. Koheil, D. McCullough, W.R. Johnson, and W.D. Fraser. Activation of complement at plasma-air or serum-air interface of rabbits. J. Appl. Physiol. 60(5):1651-1658, 1986.
41. Weisser, A., G.H.A. Clowes Jr., R.W. Colman, and R. Talamo. Sepsis and endotoxemia in pigs: A comparison of mortality and pathophysiology. In: Haberland GL, Lewis DH, eds. New aspects of trasylool therapy. Stuttgart and New York: F.K. Schattauer, Verlag, 159-174, 1973.
42. Yamada, T., P. Harber, G.W. Pettit, D.A. Wing, and C.N. Oster. Activation of the kallikrein-kinin system in Rocky Mountain Spotted Fever, Ann. Intern. Med., 88, 764, 1978.
43. Lambertsen, C.J. Relations of isobaric gas counterdiffusion and decompression gas lesion diseases. In: Vann, R.D., Ed. The Physiological Basis of Decompression: UHMS Publ. 75 (Phys) Bethesda, MD: Undersea and Hyperbaric Med. Soc., 87-106, June 1989.

APPENDIX D

APPENDIX D.

PRIOR STUDIES - INFUSION OF AIR BUBBLE EMBOLI INTO RIGHT ATRIUM OF SHEEP.

Method and Measurement. Effects on Pulmonary Microvascular Ultrastructure. Relation to Pulmonary Lymph Flow.

(Extracted from Project Proposal Summary of studies conducted by Co-Principal Investigator, K. Albertine, with colleagues at University of California - San Francisco, and at the University of South Florida) (Reference (1), this Report).

Extract:

Time course of damage to pulmonary microvascular ultrastructure was related to changes in pulmonary arterial hemodynamics and lung lymph flow in anesthetized, ventilated sheep with lung lymph fistulas (Controls, one-hour air embolized, four-hour air embolized). Air bubble emboli (ca 1 mm diameter) were infused after baseline measurements into the right atrium at a steady rate sufficient to double control pulmonary arterial blood pressure. Control (non-embolized sheep) had stable hemodynamics and lymph flow throughout a six-hour experiment period.

Embolized sheep all showed qualitatively similar effects. Lymph flow increased by one hour and became more elevated at four hours. Lymph protein concentration remained constant relative to plasma protein concentration, compatible with increased microvascular permeability to plasma protein. At four hours of embolization lymph "protein clearance" (protein concentration x lymph flow) was three times the initial control value. Cardiac output was not appreciably altered by the embolism. White blood cell counts were performed, but information is not sufficient to indicate degree of a probable leucocytosis.

A systematic plan of lung biopsy was carried out at the end point of exposures, to provide for two forms of microscopic evaluation of tissue changes. Terminally the thorax was opened, the right lung hilum cross-clamped, and the left lung airways were filled with 2% glutaraldehyde-1% paraformaldehyde fixative. The unfixed right lung was used for extravascular lung water measurement and for frozen section histologic examination. The left caudal lobe was used for examination by light and transmission electron microscopy.

Frozen section histology of unfixed lung showed air bubbles throughout the lung, in the pulmonary arterial microcirculation. Extravascular lung water measurement indicated pulmonary edema by four hours of embolization. By light microscopy (glycolmethacrylate sections) air bubbles were found only in pulmonary arterial vessels (1000 to 11 m diameter). Leucocytes accumulated in lungs, and aggregated about air bubble boundaries.

Ultrastructural examination showed development of gaps between endothelial cell margins in pulmonary arterial microvessels (1000 to 100 m

diameter). Gaps were focal, with length reaching 3 m. Some endothelial cells bordering gaps showed signs of ultrastructural damage, including retraction, detachment, sloughing or necrosis. Some endothelial cell gaps were partially plugged by platelets.

Neutrophils were the predominant type of leucocyte at the blood-gas embolus interface, which was bordered by a membranous proteinaceous layer. Neutrophils and, to a lesser extent, lymphocytes became associated with this layer.

These preliminary studies involving infusions of air emboli have been reported in the Journal of Applied Physiology (J. Appl. Physiol. Respirat. Environ. Exercise Physical. 57(5): 1360-1368, 1984). They have indicated the occurrence of structural and functional changes in pulmonary microvasculature, even in the absence of states of decompression or counterdiffusion supersaturation, and any peripheral effects generated in spontaneous gas embolism.

National Aeronautics and
Space Administration

Lyndon B. Johnson Space Center
Houston, Texas
77058

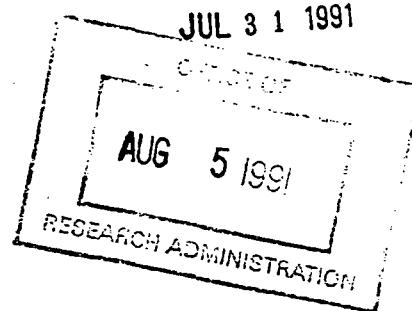


Reply to Attn of:

BE3

University of Pennsylvania
Office of Research Administration
133 S. 36th Street, Suite 300
Philadelphia, PA 19104-3426

Subject: NASA Grant NAG 9-154



The subject grant under the direction of Dr. C. J. Lambertson for "Pathophysiology of Spontaneous Venous Gas Embolism" was completed in July 1990. A final report is required to close out the subject grant. Copies of the final report should be submitted to the following addresses as soon as possible:

2 copies: NASA Johnson Space Center
Attn: J. Waligora, Mail Code SD5
Houston, TX 77058

1 copy: NASA Johnson Space Center
Attn: Nancy Kennamer, Mail Code BE3
Houston, TX 77058

2 copies: NASA Scientific and Technical Information Facility
P.O. Box 8757
Baltimore/Washington International Airport
Maryland 21240

Any questions or comments should be addressed to the contract specialist administering this grant, Nancy Kennamer, at 713-483-8522. Your prompt attention to this matter is appreciated.

Billy Jefferson
Billy J. Jefferson
Contracting Officer